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QUANTITATIVE TRAITS RELATED TO PRIMARY OPEN ANGLE GLAUCOMA IN THE SCOTTISH POPULATION ISOLATE OF ORKNEY

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DECLARATION

I declare that I composed this thesis. It is my own work. Where others have played a role, their contributions have been acknowledged accordingly. This work has not been submitted for any other degree or professional qualification.

Vidarshi Karunaratne
September 2010

DEDICATION

This work is dedicated to my husband, Denes, and my parents, Ammi and Appachchi

TABLE OF CONTENTS

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ABSTRACT

The aetiology and pathogenesis of primary open angle glaucoma (POAG), the second most common cause of irreversible visual loss in the United Kingdom, remains a conundrum for contemporary ophthalmology. Evidence suggests that glaucoma is a complex disorder, where multiple genes interact with each other and with factors in the environment. However, the aetiological heterogeneity of glaucoma coupled with its varied clinical presentation and course has made the study of glaucoma genes problematic. We established the Orcades Eye Study, a cross sectional family based genetic study, to explore the inheritance of primary open angle glaucoma (POAG). As POAG is a disease of late onset and low prevalence, rather than study disease per se we chose to study quantitative traits (QTs) related to POAG, in an isolated population in the northern Scottish archipelago of Orkney. A number of factors in this population, including reduced genetic heterogeneity and more homogenous environmental effects, confer certain advantages over more admixed urban populations in complex disease gene mapping. Preliminary analysis of the procured quantitative trait data (n=256) has demonstrated that the values obtained for the POAG related QTs of intraocular pressure (IOP), central corneal thickness and a number of optic disc parameters including optic cup area, disc area, retinal nerve fiber thickness, vertical cup to disc ratio and peripapillary atrophy are not dissimilar to other published White Caucasian populations. We also found that intraocular pressure shows an increase with age and is influenced by central corneal thickness but found no relationship between IOP and gender or IOP and other ocular biometric variables including optic nerve head parameters and refractive components. Neither central corneal thickness nor optic nerve head parameters had a statistically significant relationship to age, gender or other tested ocular biometric parameters. These findings are clinically important as these factors should be taken into consideration when evaluating intraocular pressure and other ocular biometric traits in the investigation of glaucoma and other ocular diseases in the population of Orkney. Data collection is ongoing, and with time, an increased sample size and a meaningful genetic analysis, the Orcades Eye Study will hopefully identify genes and regions of the genome associated with primary open angle glaucoma susceptibility in the Scottish Population Isolate of Orkney. To our knowledge, the only other population based study which has investigated as large a number of glaucoma related QTs is the Beijing Eye Study.

LIST OF TABLES AND FIGURES

TABLES

FIGURES

ABBREVIATIONS

ACD	Anterior Chamber Depth
AGE	Advanced Glycation End (product)
AL	Axial Length
AX	Axis
BDNF	Brain-derived Neurotrophic Factor
CABG	Coronary Artery Bypass Graft
CCT	Central Corneal Thickness
CDR	Cup Disc Ratio
CI	Confidence Interval
CNTGS	Collaborative Normal Tension Glaucoma Study
COPD	Chronic Obstructive Pulmonary Disease
CVA	Cerebrovascular Accident
CYL	Cylindrical Value
DNA	Deoxyribonucleic Acid
DVT	Deep Vein Thrombosis
EDVF	Endothelium Derived Vasoactive Factors
EPGS	European Glaucoma Prevention Study
ET-1	Endothelin-1
EUROSPAN	European Special Populations Research Network
GAT	Goldman Applanation Tonometer
GON	Glaucomatous Optic Neuropathy
GP	General Practitioner
GWAS	Genome-wide Association Study
HLA	Human Leukocyte Antigen
HPG	High Pressure Glaucoma
HRT	Heidelberg Retinal Tomograph
IBD	Identity by Descent
ICD	International Classification of Diseases
ILM	Inner Limiting Membrane
IOP	Intraocular Pressure
LED	Light Emitting Diode
LogMAR	Logarithm of the “MAR” Value
LOXL 1	Lysyl Oxidase-like 1
LREC	Local Research Ethics Committee
MI	Myocardial Infarction
MRC	Medical Research Council
N	Nasal
NHS	National Health Services
NI	Nasal Inferior
NPG	Normal Pressure Glaucoma
NPL	No Perception of Light
NS	Nasal Superior
NSAID	Non-steroid Anti Inflammatory Drugs
OAG	Open Angle Glaucoma
OCT	Optical Coherence Tomograph
OHTN	Ocular Hypertension
OHTS	Ocular Hypertension Treatment Study

ONH	Optic Nerve Head
ONHA	Optic Nerve Head Analyzer
OPTN	Optineurin
PCI	Partial Coherence Inferometry
PEX	Pseudoexfoliative Glaucoma
PL	Perception of Light
POAG	Primary Open Angle Glaucoma
PPA	Peripapillary Atrophy
PVD	Primary Vascular Dysregulation
QT	Quantitative Trait
QTLs	Quantitative Trait Loci
RGC	Retinal Ganglion Cells
RNA	Ribonucleic Acid
RNFL	Retinal Nerve Fiber Layer
SNP	Single-nucleotide Polymorphism
SNR	Signal to Noise Ratio
SPH	Spherical Value
SVD	Secondary Vascular Dysregulation
T	Temporal
TAE	Total Angular Extent
TI	Temporal Inferior
TIA	Transient Ischemic Attack
TIGR	Trabecular Meshwork Inducible Glucocorticoid Response Protein
TNF	Tumor Necrosis Factor
TS	Temporal Superior
UK	United Kingdom
VA	Visual Acuity
WHO	World Health Organization

CHAPTER 1

PRIMARY OPEN ANGLE GLAUCOMA

1.1 AN INTRODUCTION TO PRIMARY OPEN ANGLE GLAUCOMA

The solitary term "glaucoma" describes a heterogeneous group of progressive optic neuropathies that have in common, a slow progressive degeneration of retinal ganglion cells and their axons, resulting in a characteristic structural damage to the optic disc and a concomitant pattern of visual field loss if untreated (Weinreb and Khaw, 2004, Burr et al., 2007). This term is also used to describe ocular diseases which have already acquired the characteristic ocular neuropathy such as in primary open angle glaucoma (POAG), as well as other conditions in which intra ocular pressure (IOP) is elevated but no irreversible optic nerve injury has yet occurred such as in primary congenital glaucoma and acute angle closure glaucoma. It is a common disease and the primary cause of irreversible visual impairment in the world (Resnikoff et al., 2004).

There is some controversy over the true etymology of the term "glaucoma." Its origins can be traced back to ancient Greece and the works of Hippocrates of Kos. The term "glaucosis" was used to described a blinding disease which occurred most commonly in the elderly population (Fronimopoulos and Lascaratos, 1991, Tsatsos and Broadway, 2007, Lascaratos and Marketos, 1988a, Nathan, 2000). The word

"glaucoma" itself is derived from the ancient Greek word "glaukos", which can be used as a noun, an adjective or a verb and is believed to originate from the Greek verb "glausso" which means to "to glow" or to "shine". This description may perhaps relate to the inflamed eye associated with acute forms of glaucoma. The Greek word "glaukos" also means "owl" – so named because of the bird's striking, glowing eyes. Used as a noun, it can mean "blue-white" or " blue-green", which, from an ocular perspective, may refer to the blue-green hue sometimes associated with corneal oedema or cataract.

Whatever its true etymology, the term "glaucosis" of antiquity is unlikely to have represented the group of diseases that is described today under the term of "glaucoma". There is evidence to suggest the original term was used to described cataracts rather than a disease associated with raised ocular pressure (Fronimopoulos and Lascaratos, 1991, Tsatsos and Broadway, 2007). At the time of Hippocrates of Kos (460-377 BC), there was little differentiation between the two conditions, all afflictions of the eye being attributable to "disturbed or ill humours" (Cohen, 2001). The initial differentiation between these two entities is credited to the Romans, initially Celsus (25 BC– 50AD) and subsequently Galen (131-210AD) who described cataract as potentially amenable to remedy and glaucoma as incurable (Cohen, 2001).

Early medical records of glaucoma consisted of isolated observations by a number of medical practitioners, with references being made to what would now be considered advanced angle closure glaucoma. The Arabian surgeon Al-Tabari in the 10th century

is the first known individual to describe an association between raised intra-ocular pressure (IOP) and glaucoma (Cohen, 2001). Four centuries later Sams-ad-Din of Cairo described a certain ocular malady as " migraine of the eye……pain in the eye…dilation of the pupil and cataract. If chronic …blindness intervenes." Following the post-mortem examination of eyes, Brisseau was the first to propose glaucoma was not associated with a lens disorder. He suggested that the term *cataract* should be reserved for opacities of the lens and that glaucoma was secondary to an abnormality of the vitreous (Nathan, 2000, Drews, 2006). About a century later , the British surgeon James Guthrie, coined the term "glaucoma" to describe an ocular disease associated with rigidity of the globe. Table 1.1 summarizes some of the milestones in the history of glaucoma.

TABLE 1.1: SELECTED MILESTONES IN THE HISTORY OF GLAUCOMA[1]

YEAR	INDIVIDUAL(S)/TEAM CREDITED WITH DESCRIPTION/DISCOVERY	DESCRIPTION/DISCOVERY
c.460-777BC	Hippocrates	Uses the term "glaucosis" to describe a blinding eye condition.
c.330-260/255BC	Herophilus, Erasistratus	"nerve channels" described in optic nerve
25BC-210AD	Celsus (25BC-50AD) Galen (131-210AD)	Initial differentiation between glaucoma and cataract. "Psychic pneuma" circulated via central and peripheral nervous systems to provide body with sensation (Galen). Lens believed to be responsible for vision and nourished by retinal blood supply. Described channels in optic nerve – "poroi optika."Optic nerves believed to join at chiasma to produce single vision.
~10th century	Al-Tabari	Relationship between raised eye pressure and glaucoma described
c.1514-1600	Versalius, Fallopia, Coiter	Finds little evidence to support Galen's theory of optic nerve channels (Versalius). Organization of nerves reported in the term of "fibres" (Fallopia and Coiter), but nerve remains structure through which substances flowed, hence nerve fibers still considered hollow.
1573	Varolio	Credited with first detailed recorded dissection showing the structure of the optic nerve and its relationship to the central nervous system.
1626	Bannister	Digital tonometry to identify elevated IOP
1664	Descartes	Develops working model of optic nerve further. Describes nerve as a cylindrical structure enfolding bundles of smaller cylinders which contains slender filaments which appear to arise from the brain.
1668	Mariotte	Reports on the presence of a blind spot in the visual field corresponding to the position of the optic nerve head.
1707	Brisseau	Further differentiation between glaucoma and cataract. Lens identified as site of cataract formation
1717	Van Leeuwenhoek	Microscopic examination of the optic nerve provides further evidence for Descartes' description of optic nerve structure. Also unable to find evidence for Galen's poroi optika. Suggests once the eye visualizes an object, "globules" carry the image to the brain.
1738-1826)	Platner (1738) Demours(1818) Guthrie (1823) Weller (1826)	Several texts published concurrently regarding the importance of "the hardness of the eye" as a symptom Guthrie credited with coining the term "glaucoma"
1741	St.Yves	Periods of blurred vision may occur prior to signs of advanced glaucoma

[1] Information summarized from the following references: LASCARATOS, J. & MARKETOS, S. (1988b) Ophthalmological lore in the Corpus Hippocraticum. *Doc Ophthalmol,* 68**,** 35-45, FRONIMOPOULOS, J. & LASCARATOS, J. (1991) The terms glaucoma and cataract in the ancient Greek and Byzantine writers. *Doc Ophthalmol,* 77**,** 369-75, NATHAN, J. (2000) Hippocrates to Duke-Elder: an overview of the history of glaucoma. *Clin Exp Optom,* 83**,** 116-118, COHEN, H. C. (2001) The History of Filtering Surgery IN MERMOUD, A. & SHAARAWY, T. (Eds.) *Non-Penetrating Filtering Glaucoma Surgery* London, Martin Dunitz Ltd, DREWS, R. C. (2006) Green cataract. *Arch Ophthalmol,* 124**,** 579-86, TSATSOS, M. & BROADWAY, D. (2007) Controversies in the history of glaucoma: is it all a load of old Greek? *Br J Ophthalmol,* 91**,** 1561-2. KRONFELD, P. C. & (2009) The History of Glaucoma. IN TASMAN, W. & JAEGER, E. A. (Eds.) *Duane's Ophthalmology.* 530 Walnut Street, Philadelphia, Pennsylvania 19106-3621 Lippincott Williams & Wilkins.

1791	Galvani	Proposes “electric fluid” rather than previously postulated spirits and fluids important in the function of the nervous system
1801-1896	Longet, Muller, Bois-Reymond	Further work into the electric nature of nerve conduction. Electricity believed to produce a “nerve principle”. Presence of electric currents within nerves proven (Bois-Reymond, 1843).
1830-1854	Mackenzie	Makes distinction between acute and chronic forms of glaucoma. Describes different stages of chronic glaucoma. Suggests scleral puncture as a method of treatment.
1851	Helmoltz	Introduces the ophthalmoscope into clinical practice.
1854	Von Jaeger Von Graefe	Optic disc evaluated with ophthalmoscope Initially disc described as a swelling as opposed to cupping
1855	Weber	Clarifies optic disc depressed rather than swollen
1856	Von Graefe	Credited as being the first to chart paracentral visual field defects associated with chronic glaucoma, and use these to appraise the success of surgical interventions.
1857	Kirsch, Von Graefe	Importance of evaluating optic nerve head recognized. Von Graefe’s iridectomy gains popularity as operation of choice for treating glaucoma
1857	Muller	Anatomic observation of disc cupping thought to be secondary to raised vitreous pressure on lamina
1857	Forster	First formal perimeter introduced. Initial targets of considerable size
1860s	Donders, Von Graefe, Argyll Robertson	Further classification of glaucoma. Primary and secondary forms, “glaucoma simplex”, “glaucoma with ophthalmia” Elevated pressure believed to be secondary to “hypersecretion” of intraocular liquor (Donders) Inflammation also considered important in the aetiology of raised pressure (von Graefe) Preliminary efforts at developing a mechanical tonometer (von Graefe, 1862) Describes effect of calabar bean on miosis and accommodation (von Graefe, Robertson). Miotic effect utilized in iridectomy. Experimental tonometer developed to be used on closed eyelids (1862) First mechanical tonometers trialled on human eyes (Donders 1863-68)
1862	Bowman	Suggests a 9 stage classification system (-T3 to +T3, exceptionally low to extremely high) of ocular pressure or tension based on digital palpation
1867	Weber	First applanation tonometer
1870	Knapp	Extension of blind spot described in patients with “choked discs”
1870	Stricker and others	Advancements in histology and microscopy lead to the understanding that the numbers of optic nerve fibres are reduced in diseases like glaucoma.
1870	Schwalbe	Systematically investigates aqueous flow. Believes anterior chamber communicates directly with anterior ciliary veins and is part of the lymphatic system.
1873	Leber	Disputes Schwalbe’s hypothesis. Anatomic/cannulation studies gives rise to “filtration theory of aqueous formation”. In order to maintain a steady IOP in vivo, believed that fluid outflow was accompanied by a comparable amount of fluid formation. Outflow of fluid occurred via trabecular meshwork to anterior ciliary and vortex veins.

1876-7	Laquer	Reduction of IOP noted following the use of physiostigmine in certain cases of glaucoma. Subsequently describes ability of physiostigmine to abrogate acute attacks and thwart recurrent attacks of acute glaucoma.
1877	Weber	Pilocarpine introduced as a topical hypotensive.
1879	Priestly-Smith	Believes there are vascular and nutritional as well as pressure related components to glaucomatous optic neuropathy.
1882	De Wecker	Suggests certain forms of corneo-scleral incisions would mend in a way which would allow aqueous to drain through healed tissue into subconjunctival space in the presence of a pressure gradient.
1885-88	Imbert and Fick	Investigates theory of applanation further – Imbert-Fick Law. Develops basic mechanical fixed area tonometer based on this principle. Never used on human eyes though tested on other mammals.
1885	Malakoff	Explores applanation tonometry further First known practical applanation tonometer developed. Tonometer of 1892 gains popularity
1889	Bjerrum	Further development of perimetry. 2 meter test distance introduced and smaller targets. The blind spot, the presence of relative and absolute scotomas, described. Initial seeds of the nerve fibre theory of glaucoma sown.
1889	Drance and Begg	Optic disc haemorrhage associated with glaucoma
1895	Leber	Notes increased resistance to aqueous outflow in enucleated human globes with advanced glaucoma. Development of Leber-Knies Theory describes glaucoma as a malady of abnormal aqueous outflow.
1897	Czermak	Shallow anterior chamber associated with acute inflammatory forms of glaucoma.
1899	Elschnig	Normal discs noted to be variable
Early 1900s	Trantras	Credited with being the first to examine angle of anterior chamber. Coins the word “gonioscopy”.
1902	Darier	Use of adrenaline alone or together with physiostigmine as a ocular hypotensive described.
1903	Herbert	Describes a number of operations establishing subconjunctival fistula. First report of effect of aqueous outflow on peribulbar tissues.
1905	Schnabel	Describes the development of cavities and destruction of nerve fibres. Believes mechanism of glaucomatous optic neuropathy secondary to adsorption of noxious fluid by optic nerve fibres
1905	Schiotz	Introduces indentation tonometer. Subsequently replaces Malakoff.
1906	Lagrange	Describes iridosclerotomy
1909	Freeland, Elliot	Trephine exchanged for Lagrange’s scissors
1913	Salzman	Credited as being the second pioneer of gonioscopy
1913	First international review of glaucoma surgery	Operation of choice changes from iridectomy to “filtering cicatrix” linked to the anterior chamber for chronic glaucoma. Iridectomy remains in favour for more acute cases of glaucoma.
1919-20	Koeppe	Describes method of direct gonioscopy with Koeppe lens
1920s	Seidel	Expands on Leber’s works. Describes aqueous formation in more detail, transconjunctival movement of aqueous following trephination.

1920	Curran, Seidel	Describes mechanism of acute pupillary block
1921	Pickard	Drawings of optic nerve head standardized
1922	Elliot	Optic cup enlargement and pallor associated with glaucoma
1923	Hamburger	New preparations of adrenaline introduced.
1927	Peter	Association of paracentral scotomas and chronic glaucoma described
1925-47	Tronsco	Further work on angle anatomy. Credited with clarifying the structural organization and nomenclature of the angle
1938	Barkan	Used gonioscopy routinely in clinical practice. Differentiated between “open angle” and “narrow angle” glaucoma. Suggested IOP increase in former secondary to sclerosis of trabecular meshwork. Introduced the “internal trabeculotomy” for open angle glaucoma, suggested peripheral iridectomy for narrow angle glaucoma and goniotomy for congenital glaucoma.
1954	Many	Seeds sown for the current classification of glaucoma at the International Symposium on Glaucoma
1959	Mackay, Marg	Introduces combined applanation-indentation tonometer. Forerunner of the currently widely used Tonopen.
~1960	Goldman, Schmidt	Further development of Fick’s concept of a fixed area tonometer into the now widely used Goldman Applanation tonometer
1960	Anderson, Kirsch	Cupping of the optic disc occurs before the loss of visual field
1965	Posner	Introduces Posner Applanometer, a fixed force tonometer, into clinical use.
1965-67	Draeger, Perkins	Introduce portable applanation tonometer based on the Goldman principle.
1973	Asseff, Bjerrum, Phelps, Wesimen	Vertical optic disc cupping important
1970s	Many	Well designed large scale epidemiological studies provide evidence about the risk factors for glaucoma

1.2 DEFINITION AND CLASSIFICATION OF GLAUCOMA

With increased observation and contemplation, it was gradually recognized that glaucoma was not a single disease entity but that several forms existed. Without appropriate instruments to examine the interior of the eye, glaucoma were initially classified on the basis of external appearance. The first elementary classification of glaucoma, *congestive* versus *non-congestive*, was based on the presence or absence of inflammatory changes on the surface of the globe. Classification systems that

followed co-evolved with advancement in technology and subsequent knowledge. Current methods of classification are loosely based on a system proposed in 1954 at the International Symposium on Glaucoma (table 1.2)(Consoli et al., 2005). Even today, there is no universally accepted definition or classification system for glaucoma (Kroese and Burton, 2003, Foster et al., 2002). For the purpose of this thesis, the terminology proposed by the European Glaucoma Society has been adopted. (European_Glaucoma_Society, 2003). The open angle glaucomas (OAG), as defined by the European Glaucoma Society , are group of chronic, progressive optic neuropathies, that have in common characteristic changes at the optic nerve head, retinal nerve fibre layer and are accompanied by an open anterior chamber angle. Primary open angle glaucoma (POAG) is not associated with any other ocular disease or congenital anomaly. Table 1.2 summarizes some of the different classification systems currently in use – the WHO classification of glaucoma, according to ICD-10, the American Association of Ophthalmology and the European Glaucoma Association, as well as the system proposed in 1954.

TABLE 1.2: SYSTEMS USED IN THE CLASSIFICATION OF GLAUCOMA

SYSTEM OF CLASSIFICATION	DESCRIPTION
INTERNATIONAL SYMPOSIUM ON GLAUCOMA 1954 (CONSOLI ET AL., 2005)	1. Primary Glaucoma with two sub classes (i). Simple Glaucoma (ii). Closed Angle Glaucoma (including four phases) (a). Pre-glaucoma (b). Intermittent (c). Acute (d). Chronic 2. Secondary Glaucoma: due to pre-existing disease. This may be either (i). Open angle (ii). Closed angle 3. Congenital Glaucoma: due to obstruction of drainage by congenital anomalies
WORLD HEALTH ORGANIZATION CLASSIFICATION OF GLAUCOMA ICD-10*	Diseases of the Eye and Adnexa (H00-H59) *Glaucoma* (H40-H42) H40 Glaucoma - *Excludes* ▪ absolute glaucoma (H44.5 *)* ▪ congenital glaucoma (Q15.0 *)* ▪ traumatic glaucoma due to birth injury (P15.3) H40.0 Glaucoma Suspect Ocular Hypertension H40.1 Primary open-angle glaucoma Glaucoma (primary)*(residual stage):* capsular with pseudoexfoliation of lens *chronic simple* low-tension · pigmentary H40.2 Primary angle closure glaucoma Angle-closure glaucoma (primary)(residual stage): · acute · chronic · intermittent H40.3 Glaucoma secondary to eye trauma H40.4 Glaucoma secondary to eye inflammation. H40.5 Glaucoma secondary to other eye disorders H40.6 Glaucoma secondary to drugs H40.8 Other glaucoma H40.9 Glaucoma, unspecified.

* From the World Health Organization International Classification of Diseases, ICD-10, 2007. http://apps.who.int/classifications/apps/icd/icd10online/

EUROPEAN GLAUCOMA PREVENTION SOCIETY CLASSIFICATION (EUROPEAN_GLAUCOMA_SOCIETY, 2003)	Primary Congenital Forms ▪ Primary Congenital Glaucoma ▪ Primary Infantile Glaucoma ▪ Glaucoma Associated with Congenital Anomalies (e.g. aniridia, Sturge Weber, Marfan's syndrome) Primary Open Angle Glaucomas ▪ Primary Juvenile Glaucoma ▪ Primary Juvenile Glaucoma Suspect ▪ Primary Open Angle Glaucoma (POAG)/High Pressure Glaucoma(HPG) ▪ Primary Open Angle Glaucoma Suspect (POAG/HPG Suspect) ▪ Primary Open Angle Glaucoma/Normal Pressure Glaucoma (POAG/NPG) ▪ Primary Open Angle Glauomca/Normal Pressure Glaucoma Suspect (POAG/NPG-suspect) Secondary Open Angle Glaucomas ▪ Secondary Open Angle Glaucomas Caused By Ophthalmic Conditions o Pseudoexfoliative Glaucoma (PEX) o Pigmentary Glaucoma o Lens-induced Secondary Open Angle Glaucoma o Glaucoma Associated with intraocular haemorrhage o Uveitic Glaucoma o Glaucoma Associated with Retinal Detachment o Open Angle Glaucoma Due to Ocular Trauma ▪ Iatrogenic Secondary Open Angle Glaucoma o Glaucoma due to corticosteroid treatment o Secondary Open Angle Glaucoma due to ocular surgery and laser ▪ Secondary Open Angle Glaucomas Caused by Extra-ocular Conditions - glaucoma caused by increased episcleral pressure e.g. due to orbital or retrobulblar tumours Primary Angle Closure Glaucoma ▪ Acute Angle Closure Glaucoma ▪ Chronic Angle Glaucoma
AMERICAN ACADEMY OF OPHTHALMOLOGY (American_Academy_of_Ophthalmology, 2008)	I. Open Angle Glaucomas 1. Primary open angle glaucoma 2. Normal tension glaucoma 3. Juvenile open angle glaucoma 4. Glaucoma suspect 5. Secondary open angle glaucoma II. Angle Closure Glaucomas 1. Primary angle closure glaucoma with relative papillary block 2. Acute angle closure 3. Subacute angle closure (intermittent angle closure) 4. Chronic angle closure 5. Secondary angle closure with papillary block 6. Secondary angle closure without papillary block 7. Plateu iris syndrome Childhood Glaucomas 1. Primary congenital glaucoma 2. Glaucoma associated with congenital anomalies Secondary glaucoma in infants and children

1.3 EPIDEMIOLOGY OF PRIMARY OPEN ANGLE GLAUCOMA

Recent estimates of visual impairment by the World Health Organization states that over 161 million people were visually impaired in 2002 (Resnikoff et al., 2004). Of these, 124 million people had low vision* and 37 million were blind. Glaucoma was the primary cause of irreversible visual impairment, second only to cataract in all causes. Quigley and Broman (2006) have estimated that by 2020, there will be approximately 80 million individuals with glaucoma - 59 million of these will have open angle glaucoma (Quigley and Broman, 2006). Primary open angle glaucoma is the second most common cause of blind registration after macular degeneration in the United Kingdom (Bamashmus et al., 2004) and is calculated to cost approximately £380 per patient per annum (Traverso 2005) with an estimated £300 million spent in the UK in 2002 (Rouland 2005). The epidemiology of open angle glaucoma has been the subject of many comprehensive reviews elsewhere (Leske, 2007, Leske, 1983, Leske and Rosenthal, 1979, Rudnicka et al., 2006, Pascolini et al., 2004) and will only be summarized here.

* As defined in the International Classification of Diseases and Health Related Problems, 10th Revision, Second Edition. World Health Organization, Geneva http://www3.who.int/icd/currentversion/fr-icd.htm

1.3.1 PREVALENCE OF PRIMARY OPEN ANGLE GLAUCOMA

Since as far back as the 1920s, glaucoma research has gained from a multitude of epidemiological studies, conducted across continents investigating the prevalence of open angle glaucoma. In many earlier studies, glaucoma and ocular hypertension were synonymous and failed to meet certain methodological standards such as clearly describing the criteria used to define the disease (Leske and Rosenthal, 1979, Leske, 1983). One of the first studies to be well received as being adequately designed and conducted was the Welsh Glaucoma Survey in the Rhondda Valley (Leske, 1983, Hollows and Graham, 1966). 92% of the town's eligible population were examined but only people between the ages of 40 and 75 were included in the study. The diagnostic criteria used were an IOP above 20 mmHg, cupping of the optic disc and visual field defects. The prevalence of chronic simple glaucoma was found to be 0.28% in the examined population. Since then over 50 population based studies have been conducted in different populations. These populations have been either entire towns or defined regions or by recruiting volunteers using clearly defined selection strategies such random or clustered sampling procedures. These include the Rotterdam Eye Study (Dielemans et al., 1994), Roscommon Eye Study (Coffey et al., 1993) and the Reykjavik Eye Study (Jonasson et al., 2003) from Europe, The Blue Mountains Eye Study (Mitchell et al., 1996) and Melbourne Visual Impairment Study (Wensor et al., 1998) from Australia. Studies from the United States include the Beaver Dam Eye Study (Dielemans et al., 1994), Baltimore Eye Survey (Tielsch et al., 1991), Salisbury Eye Evaluation Project, Proyecto VER (Quigley et al., 2001) and the Los Angeles Latino

Eye Study. Studies from the Caribbean include the Barbados Eye Study (Leske et al., 1994). Studies are now emerging from India (the Andhra Pradesh Eye Study (Dandona et al., 2000), the Aravind Comprehensive Eye Study (Ramakrishnan et al., 2003), Chennai Glaucoma Study (Vijaya et al., 2005), Dhaka Eye Study (Rahman et al., 2004)), Singapore (Tanjong Pagar Survey) (Foster et al., 2000)and Singapore Malays Eye Study (Foong et al., 2007) and China (Beijing Eye Study(Jonas et al., 2006), Liwan Eye Study (He et al., 2006)).

These studies show that the prevalence of POAG varies world wide and shows inter-population variation. Figure 1.1 is a summary of world-wide data, table 1.3 summarizes some of the main studies investigating the prevalence of open angle glaucoma.

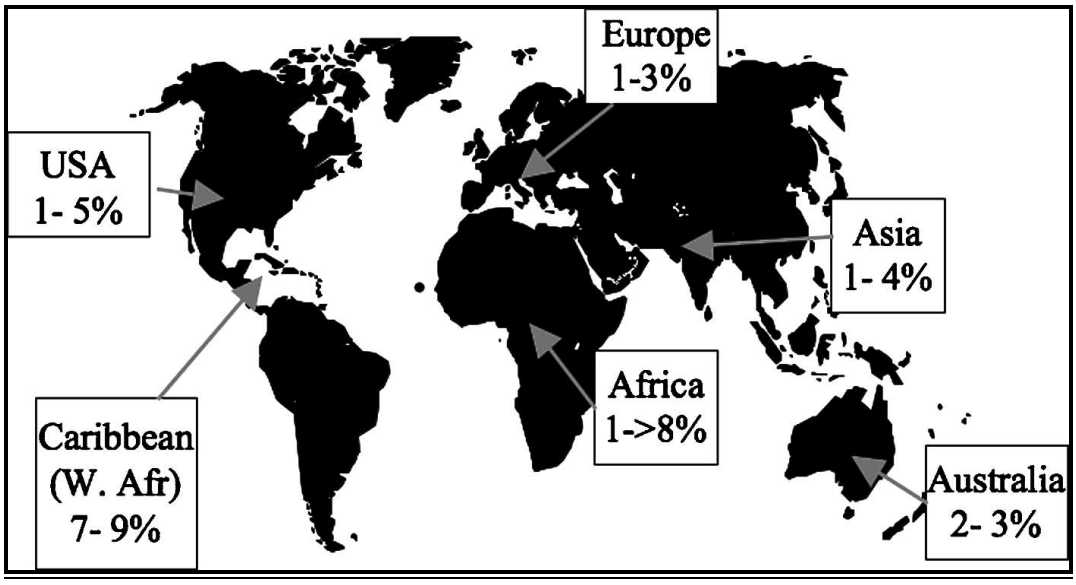

FIGURE 1.1: GLOBAL PREVALENCE ESTIMATES FOR OPEN ANGLE GLAUCOMA
(SOURCE:(LESKE, 2007)

TABLE 1.3: POPULATION BASED STUDIES INVESTIGATING THE PREVALENCE OF OPEN ANGLE GLAUCOMA 1966-2007

STUDY	YEAR	POPULATION	STUDY DEFINITION OF GLAUCOMA	AGE (years)	PREVALENCE OF OPEN ANGLE GLAUCOMA
Rhondda Valley, Wales (Hollows and Graham, 1966)	1966	Welsh n = 4,231 (examined)	▪ "Chronic simple glaucoma" : defined by the presence of 4 criteria: 1. Glaucomatous cupping of the optic disc 2. Visual field defects of a defined type 3. IOP equal or known to have been ≥21mm Hg 4. AC angle free of abnormal mesoderm and unobstructed by root of iris. ▪ "Suspected low tension glaucoma (LTG) ": As above but IOP < 21mm Hg ▪ "LTG – confirmed" : as in suspected LTG but also subsequent IOP readings < 21 mmHg. ▪ "LTG-unconfirmed"; as in suspected LTG but subsequent IOP readings in affected eyes ≥ 21 mmHg.	50-74	Chronic Simple Glaucoma: 0.28% Suspected low tension glaucoma: 0.15% Overall 0.43%
Dalby, Sweden (Bengtsson, 1981)	1981	Swedish n = 1511	▪ "Manifest Glaucoma" 1. Repeatable visual field defect consistent with glaucoma but unexplained by other ocular pathology 2. Glaucomatous optic cupping of the optic disc 3. Normal anterior segments	56-71	0.86%
St.Lucia, West Indies (Mason et al., 1989)	1989	African Caribbean	▪ "Primary"/ "Conservative" definition of glaucoma: The presence of glaucomatous visual fields found to be abnormal, by defined criteria meeting stated reliability criteria	Overall (using primary definition of glaucoma)	8.8%

CONTINUED

			▪ "Secondary" definition of glaucoma: 1. Individuals with visual fields meeting all other criteria for the definition of glaucoma but deemed unreliable by definition. 2. In the absence of visual field testing, glaucoma diagnosed if; a. IOP ≥ 30mmHg AND CDR ≥ 0.7 in either eye b. OR CD asymmetry ≥ 0.2 AND no anisometropia or other ocular disease to explain this difference.	30-39 40-49 50-59 60-69 70≤	3.9% 7.3% 8.3% 15.2% 9.5%
Baltimore Eye Survey (Tielsch et al., 1991)	1991	Black Americans White Caucasians	▪ Primary open angle glaucoma defined on: 1. The presence of glaucomatous optic nerve damage most frequently defined by characteristic visual field defects. In the absence of visual field data, glaucomatous optic neuropathy could be defined: (i) By the presence of complete cupping of the ONH AND a visual acuity ≤20/200; (ii) OR the presence of CDR asymmetry ≥ 0.4 (iii) OR in the presence of "significant" and compatible disc and nerve fibre layer abnormalities. 2. Open irido-corneal angles 3. Absence of \ secondary cause for glaucoma	40-49 80≤ 40-49 80≤	1.2% 11.3% 0.9% 2.2%
The Beaver Dam Eye Study (Klein et al., 1992b)	1992	White Caucasian n = 4926	▪ " Definite" open angle glaucoma: any two or all three of the criteria, below. Prevalences shown are of cases of definite open angle glaucoma. 1. IOP ≥ 22 mmHg in involved eye 2. Visual field defect compatible with glaucoma 3. CDR ≥ 0.8 OR difference in CDR ≥ 0.2.	43 - 84 43 - 54 75≤	2.1% 0.9% 4.7%

CONTINUED

			▪ "Probable open angle glaucoma": Individuals who did not meet the criteria for "definite" glaucoma but had a history of filtering surgery OR were on treatment for glaucoma.		
Roscommon West of Ireland (Coffey et al., 1993)	1993	White Caucasian n = 2186	Criteria used to define primary open angle glaucoma not stated explicitly but table 3 from reference lists features of glaucoma cases in the Roscommon survey: 1. Definite" glaucomatous field defect as defined in study protocol with CDR > 0.5 AND IOP ≤ 21 mmHg. 2. " Definite" glaucomatous field defect with CDR > 0.5 AND IOP > 21 mmHg 3. Visual acuity ≤ 6/60, CDR >0.8 AND IOP >30 mmHg 4. "Probable visual field defects" as described in study protocol with IOP > 30 mmHg AND CDR > 0. 5. Repeatable probable defects with CDR > 0.8 6. CDR < 0.5 with definite defect and IOP > 30mmHg Incompetent for VF defects but confirmed previous diagnosis.	Overall 50-59* 60-69 70-79 80≤	1.9% 0.7% 1.8% 3.2% 3.0%
Western Cape, South Africa (Salmon et al., 1993)	1993	Admixed population ("Cape Coloureds" – strong historical links to Indonesia, Malaysia, Madagascar, Mozambique. Also with ancestral contributions from indigenous Africans and Western Europeans).	Open angle glaucoma defined in the presence of: 1. Glaucomatous visual field loss consistent with appearance of optic nerve head 2. An open drainage angle	Overall 40-49 50-59 60-69 70≤	1.5% 0.5% 0.8% 3.0% 4.5%

CONTINUED

* Unclear if the age specific prevalence stated is for POAG or includes all types of glaucoma. POAG n=41, angle closure glaucoma n=2, secondary glaucoma n=9, ocular hypertension n = 65, glaucoma suspect n=23. Overall prevalence includes POAG cases only.

African Caribbean Eye Survey, London (Wormald et al., 1994)	1994	African-Caribbean	" Chronic glaucoma" defined in: 1. The presence of repeatable glaucomatous visual field defects in one or both eyes 2. OR in the presence of advanced visual loss which precludes the meaningful assessment of visual fields.	35≤	3.9%
Rotterdam, Netherlands (Dielemans et al., 1994)	1994	White Caucasian	Primary open angle glaucoma defined: 1. In the presence of a glaucomatous visual field defect 2. Accompanied by open and normal anterior chamber angles 3. AND with either (i). VCDR ≥ 0.5 (ii). OR CDR asymmetry ≥ 0.2 (iii). OR IOP > 21 mmHg	55 ≤ 55-59 85-89	1.1% 0.2% 3.3%
Barbados Eye Study (BES) (Leske et al., 1994)	1994	African-Caribbean White Caucasian Mixed Race	Open angle glaucoma defined in the presence of: 1. Optic disc changes as defined in study protocol (at least two signs of glaucomatous optic neuropathy including (i). A vertical or horizontal CDR ≥ 0.7 (ii). CDR asymmetry >0.2 (iii). notching of the neuro-retinal rim (iv). Most narrow area of the rim ≤ 0.1 disc diameters (v). The presence of optic nerve head haemorrhages. (vi). OR the signs of glaucomatous optic neuropathy documented in other notes accepted in the absence of photographic evidence or evidence from BES clinic examination.	40-84	7.0% 0.8% 3.3%

CONTINUED

			2. AND defined visual field changes (in the absence of reliable visual field evidence, other evidence of glaucomatous field loss such as severe impairment of vision or blindness was accepted). 3. And in the absence of other extraocular disease which may be responsible for these changes.		
Tierp Glaucoma Survey, Sweden (Ekstrom, 1996)	1996	White Caucasian	Open angle glaucoma (incuded "chronic simple glaucoma" and "capsular" glaucoma) defined on the basis of 1. A defined reproducible visual field consistent with glaucoma 2. Which could not explained by other ocular pathology or artefact.	56-74	5.9% (all OAG) 3.6% (excluding PEX)
Blue Mountains Eye Study, Australia (Mitchell et al., 1996)	1996	White Caucasian n = 4297	▪ Open angle glaucoma diagnosed in the presence of a visual field defect characteristic of glaucoma combined with either: 1. Compatible narrowing of the optic disc and CDR > 0.7 2. OR CDR asymmetry ≥ 0.3 3. AND in the absence of other ocular pathology such as rubeosis and secondary causes of glaucoma excluding pseudoexfoliation. ▪ Low pressure glaucoma defined OAG accompanied by 1. An IOP ≤21 mmHg in both eyes, 2. In subjects without a history of glaucoma surgery 3. AND not on ocular hypotensive medication. ▪ High pressure glaucoma defined as OAG accompanied by an IOP > 21mmHg.	49≤	3.0%

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Egna-Neumarkt Study, Italy (Bonomi et al., 1998)	1998	White Caucasian	▪ POAG (including patients with PEX) defined if two of the following criteria detected on second or third examination, in the presence of an open anterior chamber angle, in the absence of peripheral anterior synechie 1. IOP ≥22 mmHg 2. The presence of defined visual field defects 3. Optic nerve head findings: (i). Concentric excavation of a CDR ≥ 0.7 (ii) OR oval excavation with a difference of 0.2 between vertical and horizontal; (iii). OR Notching of the NRR; (iv). OR excavation reaching the disc margin; (v). OR disc haemorrhage (vi). Asymmetrical disc excavation with a difference in CDR>0.2 between the two eye; ▪ Normal tension glaucoma (NTG) defined as above but with IOP below 22mmHg. .	40≤	1.4% (POAG) 0.6%(NTG)
Mongolia (Foster et al., 1996)	1996	Chinese	Open angle glaucoma defined in the presence of : 1. A definite or probable glaucomatous visual field defect as defined in study protocol 2. AND a compatible optic nerve head changes 3. In the absence of an occludable angle.	40-83 60-83	0.5% 2.1%
Ponza, Italy (Cedrone et al., 1997)	1997	Italian	Primary open angle glaucoma defined: 1. In the presence of visual field defects characteristic of glaucoma and considered reliable as defined in the study protocol 2. In the absence of other ocular pathology to explain such defects	Overall 40-49 50-59 60-69 70-79 80≤	2.5% 1.4% 1.3% 2.6% 4.3% 5.1%

CONTINUED

			3. AND in the presence of an open anterior chamber angle 4. AND accompanied by at least one of the criteria below: (i) . IOP > 20mmHg (ii). OR CDR ≥ 0.5 (iii). OR CDR asymmetry ≥ 0.2.		
Melbourne Visual Impairment Project Australia (Wensor et al., 1998)	1998	Mainly White Caucasian n = 3271	No specific diagnostic criteria used to define POAG. Final classification for each individual based on using all available data for each volunteer by each expert using his or her clinical judgement to classify each case into either possible, probable or definite cases. Cases that had significant discrepancies as defined, were resolved in open discussion. Criteria for the consideration of Glaucoma Consensus Meeting 1. Past history of glaucoma 2. IOP > 21 mmHg in either eye 3. Visual field defects defined in study protocol 4. Enlarged ≥ 0.7 and or asymmetrical (≥ 0.3) CDR.	40-49 80-89	0.1% 9.7%
Tanzania, East Africa (Buhrmann et al., 2000)	2000	East African	Three different definitions of POAG used to allow comparison of POAG prevalence with other published studies. POAG defined in the absence of an occludable angle and possible secondary causes of glaucoma . ▪ Definition 1: POAG diagnosed if - 1. If CDR ≥ 0.9 2. OR CDR ≥ 0.7 accompanied by one of the following features: (i). "Definitely abnormal" nerve fibre layer (ii). OR one or at least one clock hour of complete rim loss	Overall 40-49 50-59 60-69 70-79 80≤	3.0% 1.7% 3.2% 4.7% 5.6% 4.4%

CONTINUED

			(iii). OR CDR asymmetry ≥ 0.3 in eyes if eyes had less than a 0.2 unit difference in disc diameter ▪ Definition 2: Includes all volunteers described in definition 1 but also includes the individuals with the criteria below: 1. At least one eye with a reliable visual field defect as defined in the study protocol. 2. AND CDR ≥ 0.7 3. OR a CDR asymmetry ≥ 0.2 if asymmetry is not explained by a disc diameter difference of 0.2 units using a Haag-streit micrometer. ▪ Definition 3: Includes all volunteers described in definition 1 and 2 but also includes the individuals with at least one eye with: 1. A CDR ≥0.5 2. AND a "definite, reliable" visual field as defined in the study protocol.		
Tanjong Pagar District, Singapore (Foster et al., 2000)	2000	Singaporean Chinese	In the presence of a normal drainage angle and the absence of secondary causes, POAG defined according to 3 categories: ▪ Category 1: In the presence of a visual field consistent with glaucoma, POAG defined if one of the following criteria present: 1. Eyes with CDR or CDR asymmetry ≥ 97.5th percentile for the normal population 2. OR a NRR width ≤ 0.1 CDR (between 5 and 7 o'clock or between 11 and 1 o'clock)	40 – 79	1.2%

CONTINUED

			▪ Category 2 (advanced structural damage where reliable field testing is not possible), POAG defined in: 1. Eyes with a CDR ≥ 99.5th percentile 2. OR CDR asymmetry ≥ 99.5th percentile for the normal population ▪ Category 3 (optic disc not visualized, visual field not possible), POAG defined if: 1. The visual acuity was PL or worse AND the IOP > 21mmHg 2. OR eye was defined as being blind with evidence of glaucoma filtering surgery 3. OR previous medical records confirmed previous glaucoma.		
Andhra Pradesh Eye Disease Study (Dandona et al., 2000)	2000	Indian n = 5150	▪ Primary open angle glaucoma defined: 1. In the presence of glaucomatous optic neuropathy defined as: (i). A VCDR > 0.8 (ii). OR a narrowest neuroretinal rim width < 0.2 (including classic notching) (iii). OR CDR asymmetry >0.2 between eyes 2. Coupled with a visual field defect in a location compatible with the observe optic nerve head changes. 3. In the presence of normal anterior chamber angles ▪ In the absence of visual field information, glaucoma was defined in the presence of a normal anterior chamber angles if one of the following criteria was met: 1. The presence of "significant" optic disc excavation compatible with glaucoma; 2. OR end-stage glaucoma with severe central vision loss was present 3. OR by the presence of total optic disc cupping	Overall 50-59 70≤	1.62% 2.3% 6.3%

CONTINUED

Proyecto VER (Quigley et al., 2001)	2001	"Hispanic"	Open angle glaucoma defined in the absence of an alternative explanation for disc and visual field findings, and in the presence of an open angle by gonioscopy by the: 1. The presence of a glaucomatous visual field defect as defined in the study protocol, accompanied by one of the following criteria: (i). A VCDR or asymmetry ≥ 97.5th percentile of the normal population. (ii). OR if narrowest rim width less than 0.1 by ratio to the disc diameter in a position matching the visual field defect 2. OR in the absence of a reliable visual field , the presence of a CDR ≥99.5th percentile of the normal population 3. OR in the absence of a reliable visual field AND the optic disc not visualized, glaucoma was diagnosed if (i). Visual acuity sufficiently low for the individual to be considered "legally blind" (ii). AND the IOP > 99.5th percentile for the normal population	Overall 41-49 80≤	1.97% 0.5% 12.6%
Alum-Inyi, Nigeria West Africa (Ekwerekwu and Umeh, 2002)	2002	West African	"Chronic open angle glaucoma" defined in the presence of a minimum of 2 of the following criteria in one or both eyes: 1. A VCDR ≥ 0.5 OR CDR asymmetry ≥ 0.2 2. IOP ≥ 22mm Hg 3. A visual field defect considered to be characteristic of glaucoma	30≤	2.1%

CONTINUED

KwaZulu-Natal, South Africa (Rotchford and Johnson, 2002)	2002	South African (Zulu)	" Definite open angle glaucoma" defined in the absence of an alternative explanation for disc and visual field findings and in the presence of an open angle by gonioscopy: 1. A glaucomatous visual field defect as defined in the study protocol accompanied by either: (i). VCDR ≥ 97.5th percentile of the normal population. (ii). OR VCDR asymmetry ≥ 97.5th percentile of the normal population. 2. OR in the absence of of a reliable visual field, a CDR ≥ 99.5th percentile of the normal population. 3. OR in the absence of a reliable visual field AND the optic disc not visualized, glaucoma was diagnosed if the visual acuity ≤ PL AND IOP > 30mmHg.	Overall 40-49 50-50 60-69 70-79 80≤	2.8% 1.2% 1.9% 2.8% 4.9% 7.7%
Bangkok, Thailand (Bourne et al., 2003)	2003	Thai n=701	Glaucoma defined , according to 3 categories , n the absence of secondary causes and the lack of alternative explanations visual defects in categories 1 and 2: ▪ Category 1: Glaucoma defined in eyes in the presence of a visual field consistent with glaucoma if one of the following criteria present: 1. With CDR ≥ 97.5th percentile for the normal population 2. OR CDR asymmetry ≥ 97.5th percentile for the normal population ▪ Category 2: Advanced structural damage where reliable field testing is not possible, glaucoma defined in eyes with: 1. CDR ≥ 99.5th percentile for the normal population 2. OR CDR asymmetry ≥ 99.5th percentile for the normal population	50≤	2.3%

CONTINUED

			▪ Category 3: Optic disc not seen because of media opacities and visual fields not possible, glaucoma defined if: 1. VA < 3/60 AND the IOP > than 99.5th percentile 2. OR VA < 3/60 AND the eye shows evidence of glaucoma filtering surgery. Gonioscopy performed but open angles or degree of open angle not defined for POAG.		
Reykjavik Eye Study, Iceland (Jonasson et al., 2003) ***CONTINUED***	2003	White Caucasian	Diagnostic criteria for open-angle, one for the following: ▪ Category 1: Two out of 3 of the following criteria, in the presence of a glaucomatous visual field defect, 1. VCDR ≥97.5th percentile (0.7) 2. Defined glaucomatous changes to ONH such as notching of the neuro-retinal rim 3. CDR asymmetry ≥ 97.5th percentile (≥0.2) ▪ Category 2: Two out of 3 of the following criteria, in the absence of a glaucomatous visual field defect, 1. VCDR ≥99.5th percentile (0.8) 2. Defined glaucomatous changes to ONH such as notching of the neuro-retinal rim 3. CDR asymmetry ≥ 99.5th percentile (≥0.3)	50≤	2.8% (OAG excluding PEX*) 4% (OAG including PEX)

* OAG- open angle glaucoma; PEX- pseudoexfoliative glaucoma,

			▪ Category 3: In the absence of a visual field test and when the optic disc can not be visualized, a subject is considered to have glaucoma is if one of the following criteria is met: 1. VA<3/60 and IOP >99.5th percentile 2. VA<3/60 and IOP >99.5th percentile and the eye show evidence of filtering surgery for glaucoma.		
Aravind Comprehensive Eye Survey (Ramakrishnan et al., 2003)	2003	Indian	Primary open angle glaucoma defined according to 2 categories: ▪ Category 1: In the presence of a reliable visual field defect compatible with one of the changes found below: 1. VCDR > 0.8 2. OR a narrowest NRR width < 0.2 3. OR CDR asymmetry > 0.2 accompanied compatible visual field defect. ▪ Category 2: In the absence of reliable visual field data, presence of one of the following was considered sufficient for the diagnosis of glaucoma: 1. "Significant" optic disc excavation in keeping with glaucomatous optic neuropathy 2. OR end stage glaucoma with severe central vision loss 3. OR total disc cupping	Overall 50-59 70≤	1.2% 1.6% 2.9%
Temba, South Africa (Rotchford et al., 2003)	2003	Black South African	In the absence of an alternative explanation for disc and visual field findings, in the presence of an open angle by gonioscopy, open angle glaucoma was defined if the criteria in one of the following categories was met:	Overall 40≤ 40-49 80≤	2.9% 0.6% 10.7%

CONTINUED

			▪ Category 1: One of the following criteria in the presence of a glaucomatous visual field defect as defined by study protocol: 1. VCDR ≥ 97.5th percentile of the normal population 2. OR CDR asymmetry ≥ 97.5th percentile of the normal population. ▪ Category 2: In the absence of a reliable visual field and the presence of one of the following criteria: 1. A CDR ≥ 99.5th percentile of the normal population. 2. OR CDR asymmetry of ≥ 0.3 ▪ Category 3: If the the optic disc not visualized, the presence of all three of the following criteria: 1. Visual acuity ≤ PL 2. AND the IOP > 30mmHg. 3. AND the presence of of an afferent pupillary defect if the pupil was visible.		
Tajimi, Japan (Iwase et al., 2004)	2004	Japanese n = 3021	In the absence of a history or findings of irido-cyclitis, or other ocular findings which were likely to cause glaucomatous optic neuropathy, POAG was diagnosed on the basis of the following: ▪ Category 1:POAG was define in the presence of a visual field abnormality compatible with associated optic disc changes and the presence of one of the following criteria: 1. VCDR ≥ 0.7 2. OR the rim width 11 to 1 o'clock or 5 to 7 o'clock is ≤ 0.1 of the disc diameter; 3. OR the differences of the VCDR ≥ 0.2 between both eyes 4. OR the presence of a nerve fibre layer defect;	Overall 40-49 50-59 60-69 70-79 80≤	3.9% 2.0% 2.7% 4.7% 8.2% 6.0%

CONTINUED

			▪ Category 2: In the absence of a reliable visual field, POAG diagnosed if: 1. CDR > 0.9 or more ; 2. OR the rim width 11 to 1 o'clock or 5 to 7 o'clock is ≤ 0.05 of the disc diameter; 3. OR the differences of the vertical CDR ≥ 0.3 between both eyes		
Ghana, West Africa. (Ntim-Amponsah et al., 2004)	2004	West African	Primary open angle glaucoma diagnosed if: ▪ Subject already on treatment for POAG OR ▪ New diagnosis made by the presence of the following criteria: 1. CDR > 0.5 ± notching with associated visual field changes. 2. OR by the presence of visual field defects either in the presence of glaucomatous optic neuropathy OR "high IOP" (possibly > 22mmHg).	Overall 30-39 40-49 50-59 60-69 70-79 80≤	8.4% 5.9% 6.3% 7.0% 8.7% 18.1% 21.2%
Dhaka, Bangladesh (Rahman et al., 2004)	2004	Indian n = 2347	POAG defined in the presence of an open angle by one of the three categories: ▪ Category 1: In the presence of a visual field defect consistent with glaucoma, and the following criteria 1. A CDR ≥ 97.5th percentile for the normal population 2. OR CDR asymmetry ≥ 97.5th percentile for the normal population with glaucoma. 3. In the absence of an alternate explanation for disc and field findings.	50-59 80 ≤	1.9% 1.1%

CONTINUED

			▪ Category 2: In the presence of advanced structural damage where reliable field testing is not possible glaucoma was defined in the presence of: 1. A CDR asymmetry ≥ 99.5th percentile for the normal population 2. OR CDR asymmetry ≥ 99.5th percentile for the normal population 3. In the absence of an alternate explanation for disc and field findings ▪ Category 3: When optic disc visualized because of media opacities, glaucoma defined if : 1. VA < 6/60 AND IOP > than 99.5th percentile 2. OR VA < 6/60 and the eye shows evidence of glaucoma filtering surgery		
Wroclaw Epidemiological Study, Poland (Nizankowska and Kaczmarek, 2005)	2005	Polish n = 4853	▪ Primary open angle glaucoma defined in the presence of: 1. An IOP > 21 mmHg 2. AND an anterior chamber angle that can not be occluded, and is absent of vascular abnormalities and goniosynechie 3. AND the following disc abnormalities (unclear if only one abnormally is required or a number of abnormalities, and only if abnormalities on ophthalmoscopy only or includes abnormalities found on optic nerve head imaging):	40≤	1.3%

CONTINUED

			(i). Rim/disc ratio <0.1 anywhere (ii). Rim/disc ratio≥0.1 and <0.4 for more than 90 degrees of disc; (iv) Oval appearance of the cup with a (v) difference in vertical to horizontal cup/disc ratio (C/D) ≥ 0.2; (iv) CDR asymmetry > 0.2 between the two eyes; (v). Presence of disc haemorrhage crossing the rim (vi). GDx discriminant analysis formula of Weinreb >0.501 or the number value >25 (vii). HRT discriminant analysis formula of Mikelbert > 0.5 4. OR in the presence of defined visual field defect compatible with optic disc findings in the absence of other ocular or neurological pathology to explain these findings. ▪ "Normal pressure glaucoma" was defined with the criteria as above except the IOP was required to be ≤ 21 mm Hg.		
Chennai Glaucoma Study, India (Vijaya et al., 2005)	2005	Indian N = 3850	Primary open angle glaucoma defined in the presence of an open drainage angle and the criteria in one of the three categories: ▪ Category 1: In the presence of a defined "definite" visual field defect consistent with glaucoma and one of the following criteria:	Overall 50-59 80≤	1.6% 1.6% 3.6%

CONTINUED

			1. CDR ≥ 97.5the percentile for the normal population 2. OR CDR asymmetry ≥ 97.5the percentile for the normal population 3. OR neuro-retinal rim reduced to ≤ 0.1 CDR between 11 and 1 o'clock or between 5 and 7 o'clock ▪ Category 2: Advanced structural damage with unproved visual loss, including subjects without reliable visual fields diagnosed with glaucoma in the presence of 1. CDR≥ 99.5th percentile for the normal population 2. OR CDR asymmetry ≥ 99.5th percentile for the normal population. ▪ Category 3: Subjects whose optic discs could not be examined e.g. due to media opacities, glaucoma diagnosed if IOP ≥ 99.5th percentile for the normal population.		
West Bengal Glaucoma Study (Raychaudhuri et al., 2005)	2005	Indian	Primary open angle glaucoma defined according to 3 categories: Category 1: POAG defined 1. In the presence of an open anterior chamber angle 2. In the absence of other ocular pathology to explain the findings below 3. AND in the absence of secondary causes for glaucoma 4. AND the presence of a VCDR ≥ 0.7 OR 5. OR VCDR asymmetry of ≥ 0.2 6. AND accompanied by a visual field defect consistent with glaucomatous optic neuropathy	Overall 50-59 80≤	3.4% 2.5% 4.3%

CONTINUED

			Category 2: In the absence of a reliable visual field, POAG defined 1. In the presence of an open anterior chamber angle 2. In the absence of other ocular pathology to explain the findings below 3. AND in the absence of secondary causes for glaucoma 4. In the presence of a VCDR ≥ 0.9 in either eye 5. OR disc asymmetry ≥ 0.3 ▪ Category 3: If the optic disc could not be examined in the presence of a media opacity, POAG was defined: 1. In the presence of an open anterior chamber angle 2. In the absence of other ocular pathology to explain the findings below 3. AND in the absence of secondary causes for glaucoma 4. AND an IOP > 26mmHg 5. AND visual acuity < 3/60 6. OR evidence of previous glaucoma surgery.		
Liwan, Guangzhou (He et al., 2006)	2006	Chinese	Primary open angle glaucoma defined according to three categories: ▪ Category 1: In the presence of a visual field defect consistent with glaucoma and the absence of a narrow drainage angle, POAG diagnosed: 1. In the presence of a VCDR ≥ 97.5the percentile for the normal population 2. OR VCDR asymmetry ≥ 97.5the percentile for the normal population	50-93	2.1%

CONTINUED

			▪ Category 2: In the presence of advanced structural damage with unproved visual loss, in the absence of reliable visual fields, and in the absence of a narrow drainage angle, POAG defined in the presence of: 1. CDR ≥ 99.5th percentile for the normal population 2. OR CDR asymmetry ≥ 99.5th percentile for the normal population. ▪ Category 3: Subjects whose optic discs could not be examined, e.g. due to media opacities had POAG defined, in the absence of a narrow drainage angle if: 1. If IOP ≥ 99.5th percentile for the normal population 2. OR are blind (VA <3/60) AND have had previous glaucoma filtering surgery		
Salisbury Eye Evaluation Glaucoma Study (Friedman et al., 2006)	2006	White Caucasians Black Americans	Subject classified as having open angle glaucoma if diagnosed as having either "definite" or probable OAG as defined below. ▪ "Definite open angle glaucoma" defined in eyes 1. With optic discs considered to have "glaucomatous appearing" optic discs 2. Accompanied with a visual field defect 3. OR the presence of total cupping. 4. In the presence of a trabecular meshwork visible for >90° 5. And the absence of peripheral anterior synechie 6. OR the presence of peripheral anterior synechie but with a history of previous ocular surgery.	73-74 75≤ 73-74 74≤	3.4% 9.4% 5.7% 23.2%

CONTINUED

			▪ Probable glaucoma: 1. "Poorer documentation" of glaucomatous visual field loss 2. But ONH had classical features of glauomca such as excavation, thin NRR, large VCDR and vertical cup disc asymmetry. 3. In the presence of a trabecular meshwork visible for >90 4. And the absence of peripheral anterior synechie 5. OR the presence of peripheral anterior synechie but with a history of previous ocular surgery		
Projecto Glaucoma Piraquara City, South Brazil (Sakata et al., 2007)	2007	White "Non-white" (black /mixed race)	Primary open angle glaucoma defined in 3 categories: ▪ Category 1: In the presence of a visual field compatible with glaucoma, an open anterior chamber angle, and in the absence of secondary causes for glaucoma, POAG defined in the presence of: 1. A VCDR ≥ 97.5the percentile for the normal population 2. OR a VCDR asymmetry ≥ 97.5the percentile for the normal population ▪ Category 2: In subjects with advanced structural damage with unproved visual loss, in the absence of reliable visual field, secondary causes for glaucoma and the presence of an open angle, POAG defined in the presence of: 1. A CDR ≥ 99.5th percentile for the normal population. 2. OR CDR asymmetry ≥ 99.5th percentile for the normal population.	40-49 50-59 60-69 70≤ 40-49 50-59 60-69 70≤	0.4% 2.8% 3.0% 6.2% 3.1% 4.0% 4.4% 4.9%

CONTINUED

			▪ Category 3: Subjects whose optic discs could not be examined , e.g. due to media opacities, in the absence of secondary causes for glaucoma and in the presence of an open angle, considered to have POAG if: 1. The visual acuity was < 20/400 AND IOP > 99.5th percentile for the normal population. 2. OR there was evidence of previous glaucoma surgery		
Skelleftea, Sweden (Astrom and Linden, 2007, Astrom et al., 2007)	2007	White Caucasian	The definition of open angle glaucoma (which included normal tension glaucoma and PEX) based on 1. The presence of visual defects consistent with glaucomatous optic neuropathy 2. In the absence of any other explanation for such defects. Though the evaluation of the disc described, it is not stated explicity that the visual field defect was required to be consistent with the appearance of the optic nerve head.	66-71 73-79 80-86 87≤	0.4% 3.5% 3.2% 4.0%

1.3.2. INCIDENCE

The numbers of studies investigating the incidence of POAG compared to the relative plethora of data on its prevalence are limited. The measurement of incidence requires a lengthy temporal follow up, and as the frequency of glaucoma in the population is relatively low, a sizeable cohort must be followed over time. The low frequency of OAG also leads to small numbers of incident cases, and hence imprecise estimates.

The first longitudinal study investigating the incidence of glaucoma using visual field testing was carried in Dalby, Sweden by Bengsston et al. who investigated the incidence of open angle glaucoma found in this population. Table 1.4 summarizes some of the population based cohort studies which have published incidence figures on open angle glaucoma. The inter-population differences in glaucoma are in keeping with the published differences in prevalence.

TABLE 1.4: POPULATION BASED STUDIES INVESTIGATING THE INCIDENCE OF PRIMARY OPEN ANGLE GLAUCOMA 1989-2007[*]

STUDY	YEAR	MAIN RACIAL GROUP	AGE (Years)	INCIDENCE OF OPEN ANGLE GLAUCOMA
Framingham, MA (Podgar 1983)[†]	1983	White Caucasian	55 60 65 70 75	0.2 0.3 0.5 0.7 1.1
Dalby, Sweden (Bengtsson, 1989) Follow up ~10 years	1989	White Caucasians	55-85	0.24%
Melbourne, Australia (Mukesh et al., 2002) Follow Up ~5 years	2002	White Caucasian	Overall 40-49 50-59 60-69 70-79 80+	0.5%[‡] 0 0.1% 0.6% 1.4% 4.1%
Rotterdam (de Voogd et al., 2005) Follow Up ~5 years	2005	White Caucasian	Overall 55-59 60-64 65-69 70-74 75-79 80+	1.2 (0.8-1.7%) 0.9% 0.5% 0.8% 1.8% 1.6% 2.7%
Barbados, West Indies (Leske et al., 2007b) ~9 year follow up	2007	African-Caribbean	Overall 40-49 50-59 60-69 70+	4.4% 2.2% 3.6% 6.6.% 7.9%

[*] All studies are prospective population based unless otherwise stated.
[†] Incidence of open angle glaucoma estimated from Framingham data.
[‡] Incidence of "definite" open angle glaucoma

Skelleftea, Sweden (Astrom and Linden, 2007) ~21 years follow up*	2007	Swedish	66 73 80 87	0.4% 3.5% 3.2% 4.0%

1.3.3 RISK FACTORS FOR PRIMARY OPEN ANGLE GLAUCOMA

The term " risk factor" describes variables that may be causal in disease as they are statistically associated with the disease, and were (or could have been) present before it's occurrence (Boland and Quigley, 2007). Risk factors for OAG have been identified from a variety of studies, from observational to experimental, and have been separated here into demographic, systemic, ocular, environmental and genetic. Table 1.5 summarizes the main risk factors with associated risk statistics.

* Mean age rather than range quoted.

TABLE 1.5: RISK FACTORS ASSOCIATED WITH OPEN ANGLE GLAUCOMA

RISK FACTOR	RISK OF GLAUCOMA (95% CONFIDENCE INTERVALS).
DEMOGRAPHIC RISK FACTORS	
AGE * **(EFFECT PER DECADE INCEASE IN AGE STRATIFIED BY POPULATION, ADJUSTED ODDS RATIO, FROM RUDNICKA ET AL., 2006)**	
African-Caribbean	1.61 (1.53 -1.70)
White Caucasian	2.05 (1.91- 2.18)
Asian	1.57 (1.46 - 1.68)
ANCESTRY (META-ANLYSIS OF PREVALANCE OF OAG STRATIFIED BY POPULATION, FROM RUDNICKA ET AL., 2006).	
African-Caribbean	4.23 (3.07-5.83)
White Caucasian	2.09 (1.61-2.70)
Asian	1.41 (1.00-2.00)
GENDER (MEN COMPARED TO WOMEN, ADJUSTED ODDS RATIO UNLESS OTHERWISE STATED, FROM RUDNICKA ET AL., 2006)	
Overall, as a prevalence adjusted for age and population	1.37 (1.22-1.53)
African-Caribbean	1.27 (1.05-1.55)
White Caucasian	1.46 (1.24-1.73)
Asian	1.36 (1.05-1.76)
OCULAR RISK FACTORS	
INTRAOCULAR PRESSURE (RELATIVE RISK, FROM SOMMER ET AL., 1991)	
≤15 mmHg	1.0
16-18 mmHg	2.0
19-21 mmHg	2.8
22-29 mmHg	12.8
30-34 mmHg	39.0
≥35 mmHg	40.1
CORNEAL THICKNESS (HAZARDS RATIO PER 40μM DECREASE , FROM GORDON ET AL., 2002)	1.7
MYOPIA (POOLED RELATIVE RISK OF MYOPES COMPARED TO NON-MYOPES, INCLUDING MYOPIA OF ALL DEFINITIONS, FROM BURR ET AL., 2007)	1.88 (1.53-2.31)
OTHER RISK FACTORS	
FAMILY HISTORY (POOLED RELATIVE RISK ESTIMATE ADJUSTED FOR AGE, FROM BURR ET AL., 2007)	3.14 (2.32-4.25)
DIABETES (POOLED RELATIVE RISK ESTIMATED, FROM BURR ET AL., 2007)	1.93 (1.38-2.69)

* From Rudnicka et al. Odds ratio from Bayesian meta-regression model. Effect per decade increase in age by population.

1.3.3.1 DEMOGRAPHIC RISK FACTORS

1.3.3.1.1 AGE

The main demographic factor associated with increased glaucoma risk is age. Increasing age has been associated consistently with increasing risk across studies, not merely observational studies but in large multi-centre randomized controlled trials such as the Ocular Hypertension Treatment Study (Gordon et al., 2002) and the European Glaucoma Prevention Study (Miglior et al., 2005, Miglior et al., 2007a). The magnitude of risk also consistently multiplies with increasing age, increasing several fold from the 40-50 year age group to the over 80 age group. (Rudnicka et al., 2006, Tuck and Crick, 1998). Other than ancestry, discussed below, no other demographic factor has been consistently associated with such increased risk. Table 1.6 summarizes the relationship between POAG prevalence, age and ancestry.

TABLE 1.6: ESTIMATED PREVALENCE OF OPEN ANGLE GLAUCOMA ACCORDING TO AGE AND ANCESTRY (SOURCE: FROM (RUDNICKA ET AL., 2006)

Age Range (Years)	Predicted Prevalence of Open Angle Glaucoma (95% confidence intervals)		
	White	Black	Asian
30-39	-	1.8 (1.2-2.7)	0.4 (0.3-0.6)
40-49	0.4 (0.3-0.6)	2.9 (1.9-4.4)	0.6 (0.4-1.0)
50-59	0.8 (0.5–1.2)	4.6 (3.1-6.8)	1.0 (0.6-1.6)
60-69	1.6 (1.1.-2.5)	7.2 (4.9-10.6)	1.6 (1.0-2.4)
70-79	3.3 (2.2-4.9)	11.2 (7.6-16.1)	2.5 (1.6-3.8)
80-89	6.6 (4.4-9.7)	16.9(11.7-23.8)	3.8 (2.3-5.9)
90-95	10.8 (7.2-15.8)	22.5(15.7-31.2)	-

Increasing age is associated with a multitude of changes to the eye and its associated blood supply – changes which may promote the pathogenesis of glaucoma. The trabecular meshwork is the key route of aqueous egress in the eye (Hart, 1992). Aqueous formed in the ciliary body, courses through the posterior to the anterior chamber, then drains through the trabecular meshwork and the uveoscleral pathways. Both pathways show a decrease in outflow with age (Gabelt and Kaufman, 2005). Trabecular meshwork cells are lost and the accrual of extracellular substances within the meshwork have been noted with increasing age. These changes may contribute to the observed age related increase in intraocular pressure observed in a number of population based studies (Klein et al., 1992a, Wu and Leske, 1997, Bonomi et al., 1998, Hashemi et al., 2005).

Glial cells of the retina and optic nerve play a multitude of roles and contribute towards many aspects of retinal ganglion cell function(Zhong et al., 2007). This includes structural and functional support for retinal ganglion cells including the provision of basic metabolic support, regulation of the extracellular environment and the production of neurotrophins. Microglia provide immune regulatory functions and assist in maintaining the perivascular barriers and attempt to protect neurones from destructive inflammatory cytokines. Aging has an unfavorable effect on the viability and regenerative capacity of microglial cells of the central nervous system (Streit, 2006). Similar age dependent attrition may be seen in glial cells of the retina and optic nerve and it possible that these changes may adversely effect their neuro-supportive and neuro-protective functions.

The retina and optic nerve of donor eyes have been demonstrated to show an increased accrual of advanced glycation end (AGE) products (Tezel et al., 2007). Though commonly associated with diabetes, AGE products are not limited to this disease. Glycation occurs concurrently with oxidation and though without abnormal glucose levels its associated changes are less marked, AGE products can be noted in relation with aging in a number of tissues. AGE related changes may contribute to biomechanical changes of the lamina cribrosa and may promote intracellular changes in retinal ganglion cells and glial cells that encourage impaired function and apoptosis.

The control of ocular blood flow is complex and involves multiple systems (Grieshaber et al., 2007). At a local level, auto-regulation attempts to meet local demands by responding to changes in perfusion pressure through a variety of mechanisms. Of particular importance are endothelial cells which not only serve an important barrier function, but also have a role in controlling vascular resistance by functioning as a central hub for the collection, integration and subsequent response to a variety of physical and biochemical stimuli such as mechanical stress and oxygen tension. The appropriate endothelium derived vasoactive factors (EDVFs) are released in response to various stimuli and these collaborate with other systems such as the autonomic nervous system to produce a suitable response. The key components are nitric oxide, which causes vasodilation by stimulating the relaxation of pericytes and smooth muscle cells via cyclic guanine monophosphate, and endothelin-1, which has the converse effect on vascular tone via endothelin receptors which increase intracellular calcium levels. Excessive concentrations of ET-1 have been demonstrated to cause vasospasm. Aging is associated with a number of changes to the vascular system including thickening of the

intima and media and increased rigidity of the vascular wall, an increased response to vasoconstrictors and deterioration in endothelial function. (Yildiz, 2007). With age, endothelial cells demonstrate increasing levels of apoptosis and a reduced ability to regenerate. The dynamic equilibrium between vasoconstriction and dilatation, and between the pro- and anti-atherogenic systems, shifts to favour the former in both cases, with altered nitric synthase activity and a rise in the production of reactive oxygen species. These changes have obvious derogatory effects on autoregulation. Optic nerve head blood flow also declines with age (Boehm et al., 2005). All these factors may contribute to the pathogenesis of glaucomatous optic neuropathy as discussed in the sections that follow.

1.3.3.1.2 GENDER

The relationship between gender and primary open angle glaucoma is inconsistent across studies, with some prevalence studies reporting an increased frequency in men, others in women and others demonstrating little difference between studies. For example, the Baltimore and Beaver Dam studies found no significant difference in glaucoma risk between males and females (Tielsch et al., 1991, Klein et al., 1992b), Rotterdam and Barbados Eye studies found an increased risk in males(Dielemans et al., 1994, Leske et al., 1994) whilst the Blue Mountains Eye Study found an increased prevalence of POAG amongst women (Mitchell et al., 1996). Similar inconsistencies can be observed amongst incidence studies. For example, the Rotterdam and Skelleftea studies found no

significant difference between males and females in the incidence of glaucoma (de Voogd et al., 2005, Astrom et al., 2007) However, the Dalby study found a higher incidence amongst women (Bengtsson, 1989). Hence, there appears to be some evidence for a higher risk of POAG in some populations in males and in others in females.

The reasons for these for these inconsistencies are not clear. It may reflect the influence of gender in the pathogenesis of glaucoma in different populations. Gender, like ancestry, in addition to a genetic component, also has and socio-cultural implications, and it is possible that some of the differences described might be reflective of these predilections. A meta-analysis of 46 studies by Rudnicka et al. demonstrated a higher risk of POAG amongst men compared to women (Rudnicka et al., 2006). However, in certain subgroups of POAG, such as normal tension glaucoma, the prevalence is more consistently higher in women (Drance et al., 2001). There is evidence to suggest that female sex hormones may be protective against raised intraocular pressure (Altintas et al., 2004, Sator et al., 1997). Oestrogen receptors can be found in the ciliary body and outflow tract and it is possible it could influence glaucoma via aqueous formation and egress as well as vascular factors (Ogueta et al., 1999, Lee et al., 2003). Evidence from population based studies however, are inconsistent (Hulsman et al., 2001, Lee et al., 2003, Nirmalan et al., 2004).

1.3.3.1.3 ANCESTRY (POPULATION/ETHNICITY/RACE)

The third demographic risk which has been relatively consistently associated with glaucoma is ancestry. A meta-analysis of variations in the prevalence of POAG by age, gender and ancestry by Rudnicka et al (Rudnicka et al., 2006) showed that prevalence of POAG is highest amongst populations of African-Caribbean ("black") ancestry at all ages, (compared to White-Caucasian and Asian) – an estimated overall prevalence of 16% in those over the age of 70 amongst blacks compared to 6% and 3% respectively in Caucasians and Asians (Rudnicka et al., 2006). Table 1.5 summarizes the estimated prevalence of open angle glaucoma according to age and ancestry by Rudnicka et al. Not only are individuals of African ancestry at a greater risk for developing POAG, the course of the disease tends to be more aggressive and blindness is more likely to ensue in these individuals. Inter-population differences in optic nerve structure, central corneal thickness, IOP levels and the prevalence of other putative glaucoma associated risk factors such as refractive error, blood pressure, diabetes, as well as differences in response to medication, uptake of treatment have been cited as possible explanations for this differential, but these findings have not been consistent across studies (Racette et al., 2003). It's worth noting at this juncture that the classification of population diversity remains a challenging, complex and sensitive issue, with much debate over what terms such as "ethnicity" and "race" represent (Keita et al., 2004). Hence these overall figures should be interpreted with caution as using umbrella descriptions such as "Black", "White" or "Asian" oversimplifies human diversity (Tishkoff and Kidd, 2004). Individuals of African descent for example, belong to one of the most genetically varied

populations in the world. If the prevalence of POAG in populations deemed to be of "the same race" is analyzed in greater detail, there can be significant variations in POAG prevalence between populations (Kosoko-Lasaki et al., 2006). POAG prevalence is significantly lower ($p<0.001$) in South Africa, Nigeria, Tanzania and Baltimore compared to Ghana, St. Lucia or Barbados, ranging from as low as 2.9% in South Africa and rural Nigeria, to as high as 8.3% in Ghana, amongst individuals of African ancestry. Amongst "white" populations the prevalence of POAG is significantly higher in white Australians than in the Dutch ($p<0.001$).

1.3.3.2 SYSTEMIC DISEASES ASSOCIATED WITH PRIMARY OPEN ANGLE GLAUCOMA

A number of systemic diseases have been associated with primary open angle glaucoma including diabetes (Klein et al., 1994), (Dielemans et al., 1996, Mitchell et al., 1997, Bonovas et al., 2004), blood pressure (Dielemans et al., 1995, Tielsch et al., 1995b, Bonomi et al., 2000b, Mitchell et al., 2004, Leskea et al., 2004, Leske et al., 2007a), cardiovascular disease (Lee et al., 2006), thyroid disease (Lee et al., 2004a) and migraine, (Corbett et al., 1985, Phelps and Corbett, 1985, Wang et al., 1997, Drance et al., 2001). However these findings have not always been consistent across large cross-sectional population based studies as well as longitudinal trials – studies which also suggest the effects of these factors are potentially modified by the presence of other risk factors (Kahn et al., 1977b, Leibowitz et al., 1980, Klein et al., 1993, Ponte et al., 1994, Leske et al., 1995, Tielsch et al., 1995b, Tielsch et al., 1995c, Wang et al., 1997, Ellis et

al., 2000, Drance et al., 2001, Gordon et al., 2002, Geyer et al., 2003, Leske et al., 2003, Pache and Flammer, 2006, Leske et al., 2007a). These as well as a multitude of other factors – autoimmune, neurodegenerative, endocrine, vascular - are reviewed in detail by Pache and Flammer (Pache and Flammer, 2006), Flammer (Flammer and Mozaffarieh, 2007) and Grieshaber (Grieshaber et al., 2007, Grieshaber and Flammer, 2007), and more recently by Coleman and Caprioli (Coleman and Miglior, 2008, Caprioli and Coleman, Caprioli and Zeyen, 2009) and Leske (Leske, 2009). Their possible roles in the pathogenesis of glaucomatous optic neuropathy, are discussed bellow.

1.3.3.3 Ocular Risk Factors Associated with Primary Open Angle Glaucoma

1.3.3.3.1 Intra-ocular Pressure

1.3.3.3.1.1 Generation of Intra-ocular Pressure

Since the 10th century and the work of the Arabian surgeon Al-Tabari, intraocular pressure has been associated with the pathogenesis of glaucoma. (Cohen, 2001). The intraocular pressure of the eye is mainly due to the formation and outflow of aqueous humour produced by the ciliary body (Caprioli, 1992, Hart, 1992). IOP is formed by both active and passive processes, in the ciliary epithelium and circulates from the posterior to the anterior chamber of the eye via the iris. Aqueous then drains via the

trabecular meshwork and uveosecleral pathways. The former pathway, known as the "conventional route" is the main route of aqueous outflow (Llobet et al., 2003)). The latter pathway is known as the non-conventional pathway. This is responsible for only a minority of aqueous egress. Goldman's equation, $PO = (F/C) + Pv$, summarizes the relationship between IOP and aqueous inflow and outflow (Hart, 1992). PO = the IOP in mmHg, F = rate of aqueous production in micro-litres per minute (μl/min), C = facility of outflow in micro-litres per minute per millilitre of mercury (μl/min/ mm Hg), Pv is the episcleral venous pressure in mmHg.

Current paradigms suggest that the main factor that determines IOP is the outflow facility of aqueous humour, and has been reviewed extensively elsewhere (Tan et al., 2006, Tamm and Fuchshofer, 2007). In brief, the trabecular meshwork pathway provides the main route for aqueous egress from the anterior chamber (Hart, 1992, Llobet et al., 2003). This tissue is divided structurally into three regions and aqueous flows between the cells of these layers until it reaches the endothelial cells of the Canal of Schlemm. The inner layer is the uveal layer. This is formed from connective tissue arising from the iris and ciliary body stromas which is then covered by endothelial cells. This layer has large intercellular spaces and offers little resistance to aqueous outflow. The second layer is the corneoscleral meshwork which is formed from connective tissue lamellae and shows greater organization, smaller intercellular spaces and greater resistance to outflow. The layer with the greatest resistance due to it's narrow intercellular spaces and cells entrenched in extracellular matrix is the cribriform or juxtacanalicular meshwork. Aqueous flows through this resistant layer before finally crossing a lining of endothelial

cells, using both para- and intercellular routes, to enter the canal of Schlemm. In a "healthy" eye, outflow resistance is balanced by aqueous formation in the ciliary body. Pressure within the eye, intraocular pressure, rises in response to this resistance until it is sufficient to force aqueous through the trabecular meshwork into the Canal of Schlemm (Tamm and Fuchshofer, 2007). This resistance to outflow increases with age and also in various forms of glaucoma including POAG. POAG related changes found in the trabecular meshwork are not dissimilar to age related changes (Tamm and Fuchshofer, 2007).

1.3.3.3.1.2 DISTRIBUTION OF INTRA-OCULAR PRESSURE

Difference population based studies have investigated the distribution of IOP in normal and glaucomatous eyes and their findings suggest that mean IOP can vary between populations (Katavisto and Sammalkivi, 1964, Armaly, 1965, Hollows and Graham, 1966, Wallace and Lovell, 1969, Alsbirk, 1970, Kahn et al., 1977a, Kahn et al., 1977b, Shiose and Kawase, 1986, Shiose, 1990, Sommer et al., 1991, Shiose et al., 1991, Klein et al., 1992a, Dielemans et al., 1994, Mitchell et al., 1996, Leske et al., 1997, Jacob et al., 1998, Bonomi et al., 1998, Weih et al., 2001, Rotchford and Johnson, 2002, Hashemi et al., 2005, Xu et al., 2005). The mean IOP in white Caucasian adult populations, whether investigated by Schiotz tonometry or Goldman Applanation Tonometry, has consistently been estimated to being between approximately 15 to 16 mmHg, with a standard deviation of 2.5-2.8mmHg in White Caucasian populations, though standard deviations as high as 3.23 mmHg and mean IOPs of 16.8mmHg have been recorded

(Armaly, 1965, Wallace and Lovell, 1969, Burr et al., 2007). " Normal IOP " is obtained stochastically based on the mean IOP ±2 standard deviations. Hence "normal" IOP in a white Caucasian population is 15-16mmHg on average, and even though the value increases with age, 21-22mmHg is generally accepted as the upper limit of normal.

1.3.3.3.1.3 RELATIONSHIP BETWEEN INTRA-OCULAR PRESSURE AND GLAUCOMA

Strong evidence suggests IOP is intimately related to glaucoma. Population based cross-sectional studies conducted on different continents – from Beaver Dam, to the Blue Mountains to Tanjong Pagar and Tajimi to Chennai (Klein et al., 1992a, Klein et al., 1992b, Mitchell et al., 1996, Foster et al., 2003, Iwase et al., 2004, Vijaya et al., 2005) have shown that an increased IOP is associated with an increased prevalence of glaucoma. This relationship between IOP and glaucoma is a dose-dependant one (Sommer et al., 1991). Randomized clinical trials have proven that lowering IOP, whether by means of medication, surgery or argon laser trabeculoplasty, slows the onset/progression of glaucomatous optic neuropathy and visual field deterioration (Feiner and Piltz-Seymour, 2003, Heijl et al., 2002, Burr et al., 2005, Maier et al., 2005, Vass et al., 2007). For example, the Early Manifest Glaucoma Trial randomized patients with early glaucoma to observation or reduction in IOP with betaxolol and argon laser trabeculoplasty by 25%. Reduction in IOP clearly reduced the progression of glaucoma, leading to a 17% reduction in the treatment group, for a number needed to treat of 6 (Heijl et al., 2002). Furthermore, in individuals with bilateral POAG, the eye with the

higher IOP tends to lose field more quickly-this occurs even if both IOPs are <21 mmHg (Leske et al., 2003). Animal models of glaucoma have confirmed and allowed further investigation of the biological basis for this relationship (Lindsey and Weinreb, 2005, Morrison, 2005, Rasmussen and Kaufman, 2005, Weinreb and Lindsey, 2005). Overall, it is believed that raised IOP induces a series of events which eventually leads to the death of retinal ganglion cells by apoptosis (Guo et al., 2005).

1.3.3.3.1.4 MECHANISMS OF GLAUCOMATOUS OPTIC NEUROPATHY

The main mechanisms for glaucomatous optic neuropathy (GON) secondary to raised IOP (or involving raised IOP) are known as the mechanical and vascular theory of glaucomatous optic neuropathy and are reviewed in detail elsewhere (Halpern and Grosskreutz, 2002, Flammer and Mozaffarieh, 2007).

1.3.3.3.1.4.1 THE MECHANICAL THEORY OF GLAUCOMATOUS OPTIC NEUROPATHY

In brief, the mechanical theory suggests that when intraocular pressure rises above physiological levels, the pressure gradient across the lamina cribrosa increases leading to pressure induced backward bowing of the lamina cribrosa, misalignment of the pores within the connective tissue complex and subsequent deformation and mechanical stress to the retinal ganglion cells, their axons and supporting cells (Quigley et al., 1981, Quigley and Addicks, 1980c, Quigley and Addicks, 1981, Morgan et al., 1998). Retinal ganglion cells are damaged both directly and indirectly by the mechanical stress induced

by this raised pressure. This stress has been shown to obstruct both anterograde and retrograde axoplasmic flow of the optic nerve (Anderson and Hendrickson, 1974). The ability of retinal ganglion cells to survive and thrive can be seen as a dynamic balance between various factors promoting their continuing existence and other factors encouraging apoptosis and hence extinction. Obstruction of retrograde axoplasmic flow has been demonstrated to impede the movement of neurotrophic factors, for example brain-derived neurotrophic factor (BDNF) and its receptor, to the perikaryon of the retinal ganglion cell and cause the accrual of these factors at the level of the lamina cribrosa (Hollander et al., 1995, Quigley, 1995, Quigley et al., 2000, Pease et al., 2000). Deprivation of the retinal ganglion cell soma of neurotrophic factors is believed to stimulate apoptotic cell death (Halpern and Grosskreutz, 2002). Raised hydrostatic pressure has also been shown to cause the activation of glial cells, cause changes that may impair their neuro-protective and supportive function and increase the secretion of neurotoxic substances such as tumour necrosis factor alpha (Martin et al., 2002, Tezel and Wax, 2000, Yan et al., 2000). Such changes would also promote the apoptotic death of retinal ganglion cells. In addition, raised IOP can cause the atypical deposition of various forms of extracellular matrix proteins such as different forms of collagen, elastin and basement membrane material, which may alter the biomechanical, and possibly biochemical, environment of the optic nerve leading to some of the changes characteristic of glaucomatous optic neuropathy (Hernandez et al., 1990, Johnson et al., 1996).

1.3.3.3.1.4.2 THE VASCULAR THEORY OF GLAUCOMATOUS OPTIC NEUROPATHY

In the vascular theory, glaucomatous optic neuropathy is thought to arise due to impaired perfusion of the optic nerve head secondary to raised IOP and/or other factors (Halpern and Grosskreutz, 2002, Flammer and Mozaffarieh, 2007, Grieshaber et al., 2007). This reduced blood supply has been postulated to lead to oxidative stress and ischemic damage to the retinal ganglion cells, axons and their supporting cells. Cellular ischemia can lead to glutamate mediated cytotoxcity and also withdrawal of neurotrophins secondary to a reduced energy supply leading to the apoptotic cell death of retinal ganglion cells as described (Chung et al., 1999). Oxidative stress can reduce energy supplies, hamper intracellular homeostatsis of ions such as calcium, promote oxidative damage of proteins, lipids and DNA and subsequently induce neurodegeneration (Tezel, 2006). Blood flow to the ONH depends on perfusion pressure, which is defined as the difference between IOP and systemic blood pressure. Raised IOP may cause mechanical compression of ocular vasculature, effect haemodynamics and reduce the perfusion of distal tissues (Harris et al., 1996, Joos et al., 1999, Chung et al., 1999). Hence IOP has a central role to play in this paradigm as well.

1.3.3.3.1.4.3 VASCULAR DYSREGULATION AND GLAUCOMATOUS OPTIC NEUROPATHY

Factors that are IOP independent, as well as factors that are IOP dependent have been identified as potentially contributing to the advancement of damage secondary to

impaired perfusion. Vascular dysfunction, such as issues with auto-regulation or vasospasm, or abnormal vasoconstriction can also give rise to ischaemia (Chung et al., 1999, Grieshaber et al., 2007). This vascular *dysregulation* can be classified as primary or secondary (Grieshaber et al., 2007). Patients with primary vascular dysregulation syndrome (PVD) show atypical responses to certain stimuli. Patients on average, have colder hands, lower blood pressure, a reduced sensitivity to thirst, an increased sensitivity to certain medications, altered sleep patterns and sensitivity to vasoconstriction. A higher prevalence of PVD is found in women, Japanese populations and professionals compared to blue collar workers. Secondary vascular dysregulation occurs in association with other systemic conditions such as autoimmune diseases like multiple sclerosis, rheumatoid arthritis, and systemic lupus erythrematosus and infections diseases such as bacterial meningitis and acquired immune deficiency syndrome. Other conditions like certain tumours, other HLA-B27 positive diseases such as ulcerative colitis and certain drugs like interferon have also been associated with vascular dysregulation (Grieshaber et al., 2007). Only primary vascular dysregulation however is a significant risk factor for certain forms of glaucoma (Grieshaber et al., 2007). The reason for this difference is unclear but it has been postulated that the increased levels of ET-1 observed in the systemic circulation may lead to a widespread increase in vascular tone and a reduction in baseline blood flow to ocular tissue but causing little interference to local ocular autoregulation. In patients with PVD however, the response to a local reduction in perfusion secondary to physical or psychological stress appears to be impaired, hence increasing the potential for ischaemia related optic nerve injury.

The regulation of ocular blood flow requires a dynamic equilibrium between vasoconstriction and vasodilatation and involves a number of different mechanisms and factors including endothelium derived vasoactive factor endothelin-1, and nitric oxide. Patients with glaucoma have been found to have increased levels of ET-1 in their aqueous humour as well as an increased response to the inhibition of nitric oxide synthase compared to controls (Tezel et al., 1997a). In addition to it's role in vasoregulation, there is evidence to suggest that ET-1 may cause glial cell activation at the optic nerve head, disrupt axoplasmic flow, promote the apoptosis of retinal ganglion cells and induce extra-cellular matrix remodeling (Prasanna et al., 2002, Taniguchi et al., 2006, Lau et al., 2006, He et al., 2007) Other studies have demonstrated that some patients with open angle glaucoma may have a number of local circulatory issues including dysfunction of endothelial cells, deficiencies in retinal, choroidal and retrobulbar circulations and problems with autoregulation (Schwartz and Kern, 1980).

1.3.3.3.1.4.4 Oxidative Damage And Immune Factors in Glaucomatous Optic Neuropathy

What ever the initial mechanism of injury to glaucomatous tissue, subsequent oxidative stress and neuronal injury may stimulate the immune system (Tezel, 2006). Though the initial immune response may assist regeneration and repair of damaged tissue, a chronic inflammatory state may lead to further damage and deterioration. There is evidence to

suggest patients with open angle glaucoma may have increased immune activity. In one hospital based study, 30% of patients with glaucoma had one or more concurrent immune-related diseases compared to 8% of patients with ocular hypertension (Cartwright et al., 1992). Glaucoma patients have been found to have elevated levels of auto-antibodies to a number of molecules and structures including structures of the optic nerve and retina (Tezel et al., 1999, Romano et al., 1999, Joachim et al., 2005), aberrant immunoglobulins such as antibodies to DNA, RNA, nuclear antigens and monoclonal paraproteins (Wax et al., 1994) and antibodies to heat shock proteins (Wax et al., 1998, Tezel et al., 1998). Several components of the complement cascade and have been found to be up-regulated and abnormal cellular immunity has also been noted in patients with open angle glaucoma compared to age matched controls, adding further credence to the hypothesis that immune dysregulation may play a prominent role in glaucomatous optic neuropathy (Stasi et al., 2006, Yang et al., 2001). Immune related destruction may be direct such as Fas/Fas ligand interaction between primed T-cells and retinal ganglion cells or indirectly through the secretion of pro-inflammatory cytokines such as TNF-α which promotes further inflammation as well as apoptosis (Yang et al., 2001, Tezel and Wax, 2000).

1.3.3.3.1.4.5 NORMAL TENSION GLAUCOMA

In reality, the relationship between raised IOP and glaucomatous optic neuropathy is more complex than initially envisioned by ophthalmologists of the past. There is

considerable overlap between the distribution of IOP in disease-free individuals and those with POAG. Many epidemiological studies have demonstrated that glaucomatous optic neuropathy can be observed in individuals with an IOP level below the statistically defined upper limit of normal. (Hollows and Graham, 1966, Iwase et al., 2004, Klein et al., 1992b, Sommer et al., 1991). For example, in a cross-sectional epidemiological study set in Tajimi City, Japan, Iwase et al found 92% of patients diagnosed with POAG had an IOP of less than 21mmHg (Iwase et al., 2004). These individuals, who display glaucomatous optic neuropathy but with an IOP within the statistically normal range have been referred to by a variety of names – patients with "normal tension glaucoma" (NTG), "low-tension glaucoma" or "low pressure glaucoma" by some schools of thought. In other studies, the prevalence of normal tension glaucoma in the population has been found to be much less. In the Beaver Dam Study which consisted of individuals of white Caucasian ancestry, approximately a third of individuals diagnosed with definite OAG had an IOP less than 22 mmHg (Klein et al., 1992a, Klein et al., 1992b). In the Baltimore Eye Survey, 24% of OAG patients had an IOP of <21 mmHg (Sommer et al., 1991). For patients with a screening IOP of < 16 mmHg, the prevalence of OAG was 0.65%. In the baseline study group of the Early Manifest Glaucoma Trial, a randomized, controlled clinical trail which compared the effect of lowering IOP immediately versus no treatment or delayed treatment, 53.5% of patients in the treatment group and 50% of patients in the control group had baseline IOPS of < 21 mmHg (Heijl et al., 2002). IOP however, appears to be important in the progression of glaucoma even for some patients whose IOP is within the statistically normal range, as lowering IOP can reduce the progression of GON even within this group. The Collaborative Normal

Tension Glaucoma Study (CNTGS) randomized 140 eyes with normal tension glaucoma to either no treatment or medical or surgical treatment (Collaborative_Normal_Tension_Glaucoma_Study_Group_A, 1998, Collaborative_Normal_Tension_Glaucoma_Study_Group_B, 1998). A reduction of IOP by 30% lead to a significant reduction in glaucoma progression in the treatment group. However, the relationship between IOP and NTG is far from clear. Reduction of IOP does not always slow or abrogate disease progression (Collaborative_Normal_Tension_Glaucoma_Study_Group_A, 1998, Collaborative_Normal_Tension_Glaucoma_Study_Group_B, 1998, Heijl et al., 2002). 12% of patients in CNTGS with a significant IOP reduction according to protocol, continued to progress, suggesting perhaps a 30% reduction in IOP is insufficient to halt disease progression or that other factors independent of IOP are important in the pathogenesis of NTG. Furthermore, a significant percentage of patients, 65% of randomized eyes, did not show progression through the time period of the study, and curiously, untreated IOP was not identified as an significant independent risk factor for the progression of the disease despite it's effect on prevalence (Drance et al., 2001). A number of retrospective studies have attempted to investigate if asymmetric IOP leads to asymmetric visual field loss in NTG. In these studies, with patients with asymmetrical NTG demonstrated a correlation between mean IOP and asymmetric visual fields ranging from 22 to 86% of cases (Cartwright and Anderson, 1988, Haefliger and Hitchings, 1990, Crichton et al., 1989, Poinoosawmy et al., 1998). However, this finding has not been supported by all studies. For example, the Low Pressure Treatment Study, a randomized multi-centre, prospective, double blind study where NTG patients were

randomized to brimonidine tartrate 0.2% or 0.5% timolol maleate investigated the relationship between IOP asymmetry and visual field loss. Neither mean deviation nor corrected pattern standard deviation were found to be correlated to IOP (Greenfield et al., 2007).

Whether NTG should be considered a separate disease entity is a topic of great contention – a debate that has raged since as far back as the 1800s when von Graefe is said to have retracted his initial description of NTG after strong opposition of this concept from his peers (Caprioli, 1998). Opponents of categorizing OAG into high and normal pressure entities view NTG as a variant of POAG. Glaucoma patients with NTG and POAG, both show widely varying rates of disease progression and the course of the disease can not be significantly differentiated between the two. Definition for NTG over the years has also been inconsistent, particularly in studies published before 1992 (Lee et al., 1998, Sycha et al., 2003). However, opponents of the NTG concept highlight that the division between "normal" and "abnormal" IOP is almost an arbitrary one, based on an IOP value that is approximately two standard deviations above the mean IOP of a roughly normal distribution. Several studies have also found central corneal thickness in patients with NTG is on average, lower, and in patients with ocular hypertension, is on average higher, than those with POAG or individuals who are disease free (Copt et al., 1999, Morad et al., 1998). Central corneal thickness influences applanation tonometry. Hence POAG patients with thinner than average corneas are in danger of being branded as having NTG and disease-free individuals with thicker than average corneas are in danger of being regarded as having ocular hypertension. When Copt et al. corrected for

corneal thickness in their study, a third of patients with NTG were reclassified as having POAG and over a half of patients initially thought to have ocular hypertension were re-classified as normal (Copt et al., 1999).

Furthermore, like many biological parameters, IOP also has a circadian rhythm, and can show daily fluctuations ranging from 3 to 6 mmHg, with a fluctuation greater than 10mmHg being deemed pathological (Katavisto, 1964, Kitazawa and Horie, 1975). IOP shows diurnal variation, being higher in the early morning and later in the day. However, nocturnal IOP has been found to be lower than diurnal IOP when measured in the sitting position but is higher than diurnal IOP when measured supine. In one report, two thirds of glaucoma patients and over 90% of healthy controls had IOP peaks during the nocturnal period (Mosaed et al., 2005). There is also evidence that IOP can also show seasonal variation (Bengtsson, 1972, Blumenthal et al., 1970, Qureshi, 1996), postural variation (Anderson and Grant, 1973, Jain and Marmion, 1976), have variations associated with refractive error, ordinary blinking and hormonal changes (Qureshi et al., 1996). IOP may also be influenced by a variety of systemic factors including systolic blood pressure and body mass index (Bengtsson, 1972). Hence a single IOP measurement during daytime, is a mere snapshot of the intra-ocular pressure of the eye at that moment in time, and may not reveal it's true profile (Weinreb and Liu, 2006). A diurnal curve established by taking several measurement at different times over the course of several days may provide more information but still only estimates a small proportion of possible variation. Hence, using a single or even multiple measurements

of IOP to use as the sole criterion to differentiate between two forms of open angle glaucoma and establish a different disease entity may not be a sound philosophy.

Proponents of NTG as a separate disease entity from POAG argue that there is some evidence that NTG patients have a different clinical course as certain clinical features are more commonly associated with NTG patients compared with POAG patients. For example, some studies that have shown that optic discs in NTG more commonly show shallow cupping or saucerization, with a thinner neuro-retinal rim area, especially in the infero-temporal pole (Caprioli and Spaeth, 1985, Fazio et al., 1990). A higher prevalence of optic disc haemorrhages have also been associated with NTG compared to POAG (Kitazawa et al., 1986, Sugiyama et al., 1997, Jonas and Budde, 2000). Visual field loss in NTG compared to POAG has been described as deeper, steeper and more likely to be paracentral (Caprioli and Spaeth, 1984, Hitchings and Anderton, 1983). Certain factors which were mentioned whilst discussing the pathogenesis of glaucomatous optic neuropathy such as cardiovascular risk factors (Goldberg et al., 1981, Waldmann et al., 1996) evidence of vascular dysregulation such as an increased prevalence of optic disc haemorrhages (Kitazawa et al., 1986, Healey et al., 1998), a decrease in capillary nail-fold blood flow velocity and reduced finger blood flow following exposure to the cold (Gasser and Flammer, 1991, Drance et al., 1988), greater nocturnal hypotension (Kaiser et al., 1993, Hayreh et al., 1994, Meyer et al., 1996) as well as an increased prevalence of conditions associated with vasospasm such as migraine (Phelps and Corbett, 1985, Broadway and Drance, 1998, Drance et al., 2001, Cursiefen et al., 2000) are found more consistently in patients with NTG than high tension glaucoma. Some studies have found

endothelin-1 levels tend to be higher in NTG patients compared to patients with high tension glaucoma (Cellini et al., 1997, Sugiyama et al., 1995) Female gender (Orgul et al., 1995) and a wide variety of immune factors such as an increase in the prevalence of immune-related diseases (Cartwright et al., 1992), abnormal antibodies and other immune phenomena (Wax et al., 1994, Wax et al., 1998, Tezel et al., 1998, Tezel et al., 1999, Yang et al., 2001) are also often more frequently associated with NTG (Kiuchi et al., 2006). These findings have not been supported by all studies (Carter et al., 1990, Demailly et al., 1984, Kaiser et al., 1995). Many of these factors, as discussed above, are believe to contribute to glaucomatous optic neuropathy either synergistically or in an IOP independent way.

1.3.3.3.1.4.6 OCULAR HYPERTENSION

In addition to a population of people with glaucomatous optic neuropathy without raised pressure, a population of individuals exist, who consistently show an IOP above the normal range but fail to develop glaucomatous optic neuropathy. This phenomena, is known as ocular hypertension. Ocular hypertension is 10 times as common as glaucoma and only a minority of ocular hypertensives progress to develop glaucoma (Kitazawa et al., 1977, Gordon et al., 2002). The Ocular Hypertension Treatment Trial, OHTS, a large, multi-centre controlled clinical trial, randomized 1636 patients with ocular hypertension to either observation or treatment. Ocular hypertension was defined as an IOP between 24 and 32 mmHg in one eye and between 21 and 32mmHg in the other eye, normal visual fields and optic discs and open drainage angles on gonioscopy. At

approximately 5 years follow up, only 4.4% in the treated group and 9.5% in the observation group had developed POAG. The European Glaucoma Prevention Study, a multi-centre, randomized, double-blind controlled clinical trial also investigated if the onset of POAG could be prevented or delayed in patients with ocular hypertension by treatment (Miglior et al., 2007a, Miglior et al., 2007b, Miglior et al., 2005). The inclusion criteria differed slightly from OHTs - IOP had to be between 22 and 29mmHg in at least 1 eye on 2 consecutive measurements taken at least 2 hours apart. Patients were randomized to either a placebo or dorzolamide. Only 13.4% of treated patients and 14.1.% of patients on placebo developed POAG. Despite these differences in results both OHTs and EPGS identified IOP as an important risk factor for the progression of ocular hypertension to POAG. Even though EPGS could not demonstrate a significant difference in results between treating with placebo versus dorzolamide, a 1 mm Hg baseline higher IOP was associated with an 18% higher risk of progressing to POAG. A review and meta-analysis of different randomized controlled trials have shown that lowering IOP in both ocular hypertension and manifest glaucoma reduces the risk of long term visual field loss (Maier et al., 2005, Vass et al., 2007).

1.3.3.3.1.4.7 More Comprehensive Paradigms of Glaucomatous Optic Neuropathy

The existence of ocular hypertension and normal tension glaucoma, suggests there are different populations of individuals where IOP has a different effect on the optic nerve

head. Hence the relationship between IOP and glaucomatous optic neuropathy is not as straightforward as previously thought. However, though IOP is now no longer considered a prerequisite for the diagnosis of glaucoma, together with age, it still remains one of two major risk factors, as well the only treatable component of the disease (Suzuki 2006, Friedman 200d4, Gordon 2002). A more comprehensive paradigm which embraces and explains the existence of primary open angle glaucoma, ocular hypertension and normal tension glaucoma would be to view glaucomatous optic neuropathy as the effect of IOP on a *susceptible* optic nerve head – depending on the vulnerability of the optic nerve head the effect of a given IOP would differ (Burgoyne et al., 2005). An IOP which is physiological to one optic nerve head will be pathological to another. In addition, in some cases of glaucomatous optic neuropathy, IOP dependent mechanisms may predominate whereas in others, more IOP independent mechanisms such as immune mediated processes may dominate.

1.3.3.3.1.4.8 BIOMECHANICAL MODELS OF GLAUCOMATOUS OPTIC NEUROPATHY

Glaucoma is an optic neuropathy that is characterized by the progressive degeneration of both the retinal ganglion cells and their axons (Weinreb and Khaw, 2004). Clinically this is characterized by glaucomatous excavation of the optic nerve head. Though there may be important contributions to glaucomatous optic neuropathy from other sites of damage such as within the photoreceptors (Janssen et al., 1996, Panda and Jonas, 1992), the retinal ganglion cell stroma, the lateral geniculate body and the visual cortex (Yucel et al., 2003, Yucel et al., 2000, Yucel et al., 2001, Gupta and Yucel, 2001, Gupta and

Yucel, 2003, Gupta and Yucel, 2007c, Gupta and Yucel, 2007b, Gupta and Yucel, 2007a, Gupta and Yucel, 2006, Gupta et al., 2006, Gupta et al., 2008), pioneering work by Harold Quigley and associates suggest that the initial site of damage in glaucoma occurs at the lamina cribrosa of the optic nerve head (Quigley and Addicks, 1980a, Quigley and Addicks, 1980b, Quigley et al., 1982, Quigley and Addicks, 1981, Quigley et al., 1983). The lamina cribrosa and its associated blood supply, and supporting tissue, is the Achilles heel of an otherwise sturdy corneo-scleral coat. In higher primates the lamina cribrosa consists of a three dimensional network of flexible byzantine beams of connective tissue located at the centre of the optic nerve head. Encased within these beams and within the adjacent sclera run the intra-laminar and inter-scleral capillaries from the short posterior ciliary arteries. The lamina cribrosa supports the retinal ganglion cells both structurally and functionally as their axons exit the globe via its complex pores from the relatively high pressure environment of the intraocular space to a comparatively low pressure environment of the retrobulbar compartment. The scleral-corneal coat creates a relatively closed structure around the ocular contents, helping to maintain a stable shape which is vital for the optical properties and function of the eye. Under physiological conditions, this intraocular pressure is maintained by a complex equilibrium between aqueous humour formation and subsequent escape thought the trabecular meshwork and uveo-scleral pathways (Hart, 1992). IOP generates a force within the eye and the eye wall. Mathematical and engineering methods, in particular finite element modeling, have been used to describe the effect of IOP on ocular tissue whilst defining the optic nerve head as a biomechanical structure (Burgoyne et al., 2005). Within these paradigms, the ocular coats, including the optic nerve head, are seen

as being constantly exposed to a certain level of IOP related *stress*, which is a certain force per unit cross sectional area. This is vital in order to maintain a stable shape for the globe. Stress can consist of simple forces of tension or compression, or be more complex such as shear, hydrostatic or biaxial (for a discussion and review of these definitions see Ashby and Jones (Ashby and Jones, 1984)). A certain *stress* generates a certain *strain*, which is the deformation of the tissue in response to stress. Bellezza, Hart and Burgoyne proposed the tissues of the optic nerve head -load bearing tissues such as the peripapillary sclera, scleral canal and lamina cribrosa as well as neuroretinal tissue, associated supporting cells and vasculature, are constantly exposed to IOP related stress, even at physiological levels of IOP (Bellezza et al., 2000). In this biomechanical model, the effect of IOP related stress on ocular tissues depends not merely on the level of IOP but on the susceptibility or the individual properties of the optic nerve head experiencing this force. This paradigm embraces and encompasses both the "mechanical" and "vascular" models, abrogating any need for what is essentially an unnatural separation (Burgoyne et al., 2005, Van Buskirk and Cioffi, 1993, Van Buskirk and Cioffi, 1992). It also explains the existence of both high and normal pressure varieties of primary open angle glaucoma as well as ocular hypertension - the three entities being part of a spectrum of similar pathologic processes, with optic nerve heads of varying vulnerability, requiring different levels of IOP to cause glaucomatous damage.

Bellazza, Burgoyne et als' paradigm proposes that the effect of the stress and subsequent strain induced at a certain IOP, is dependent upon the response of that tissue

that experiences it. Stress and strains that are deemed “physiological” for an individual tissue will provoke changes in surrounding tissues that are considered a part of normal aging (Burgoyne et al., 2005). Pathological levels of stress will stimulate changes that hasten the pattern of disease that is recognized as glaucomatous optic neuropathy. As the susceptibility of the optic nerve head to IOP increases, the level of IOP required to induce glaucomatous optic neuropathy decreases. Levels of IOP which are physiological to one optic nerve will be pathological to another. The effect of a certain IOP generated stress on the optic nerve depends on the biomechanics of the individual optic nerve head, which in turn is influenced by its material properties and geometry (anatomy). The material properties of a tissue describes its ability to resist deformation under an applied load – it can be considered to be the compliance or stiffness of a particular material. Materials are often described as linear or non-linear depending on the association between load and deformation. Non-linear materials do not have a constant Young’s Modulus as their relationship between load and deformation is not constant. Biological soft tissue such as the sclera tend to be non-linear, anisotropic viscoelastic materials (Ethier et al., 2004, Downs et al., 2003, Downs et al., 2005). Hence sclera shows an increased resistance to strain at higher loads. If the sclera is loaded in one direction, the initially gathered collagen fibres embedded in their extra-cellular matrix gradually unfold. This unfurling of collagen fibres is probably responsible for its nonlinearity, and its fibre orientation responsible for its anisotropy.

The second factor that contributes to ONH biomechanics is the geometry of the lamina cribrosa and sclera – the form and size of the scleral canal, the density of peripapillary

sclera, configuration and thickness of the collagenous beams that form the lamina. Three dimensional biomechanical models of the optic nerve head and surrounding sclera investigating the effect of geometrical variation on stress, have demonstrated that the highest stresses occur where the optic canal has a small radius of curvature and where the peripapillary sclera is thinnest. Considerable attention has been focused on the biomechanics of the lamina cribrosa and surrounding area (Bellezza et al., 2000, Burgoyne et al., 2004, Burgoyne et al., 2005, Edwards and Good, 2001, Ethier et al., 2004, Hernandez, 2000, Levy and Crapps, 1984, Levy et al., 1981, Morgan et al., 2002, Morgan et al., 1995). There is evidence to show that retro-displacement or "backward-bowing" of the slender lamina induced by increased IOP, can lead to structural damage of the ganglion cell axons and the blockage of axoplasmic flow as axons pass through the misaligned lamina sheets (Quigley and Anderson, 1976, Levy and Crapps, 1984, Quigley et al., 1983, Anderson and Hendrickson, 1974, Minckler et al., 1977). It is hypothesized that damage to the optic nerve occurs via compression, shearing or distension of the retinal ganglion cells as they exit the globe via the lamina cribrosa (Sigal et al., 2007) as well as damage to other supporting tissue such as astrocytes, endothelial cells and their supporting pericytes by IOP lead stress and strain. The damage incurred and the susceptibility of the ONH will depend on not merely the pressure but the biomechanical reaction of the vascular supply and tissues of the optic nerve head, and the subsequent oxidative stress and cellular response to a given pressure. The more susceptible the optic nerve, the lower the pressure required to damage it.

Since the 1970s, an increasing amount of evidence has suggested a relationship between peripapillary atrophy (PPA) and glaucoma (Primrose, 1970, Wilensky and Kolker, 1976, Nevarez et al., 1988, Buus and Anderson, 1989, Jonas et al., 1992, Jonas and Xu, 1993, Jonas et al., 1989, Jonas and Naumann, 1989, Araie et al., 1994, Jonas et al., 2002, Lee et al., 2002, Xu et al., 2007c). Histologically PPA is characterized by areas of varied pigmentation and chorio-retinal atrophy in what is known as alpha zone PPA, and a reduction in the numbers of photoreceptors and loss of retinal epithelial cells in the case of beta-zone PPA (Fantes and Anderson, 1989, Kubota et al., 1993, Curcio et al., 2000). This biomechanical paradigm provides a possible explanation for this association – factors of the surrounding peripapillary area may mean this area is more vulnerable to IOP (or other factor) induced PPA, the presence of which may in turn cause the optic nerve to be even more susceptible to damage at certain IOPs. The optic nerve head shows both great inter and intra-individual variation (Dandona et al., 1990, Sing et al., 2000). These individual differences in material properties and geometry helps account for differences in optic nerve head susceptibility to differing levels of IOP. Hence certain combinations of tissue geometry, rigidity and perfusion may be more susceptible to damage at levels of IOP within the "normal" range, whereas others with a more robust combination of these factors will be able to withstand higher levels of IOP lead stress and strain. Inter-population difference in optic nerve head morphology may help account for the variation in susceptibility amongst different populations to open angle glaucoma (Racette et al., 2003).

1.3.3.3.2 CENTRAL CORNEAL THICKNESS

1.3.3.3.2.1 THE STRUCTURE OF THE CORNEA

The cornea could be considered a transparent extension of the more opaque sclera and a part of the anterior segment of the globe (Pepose and Ubels, 1992). It is multifunctional. It acts as a barrier between the anterior chamber of the eye and the external world and also refracts and transmits light to the posterior pole. To aid these functions its five layered structure is highly specialized and combines high tensile strength and transparency. Central corneal thickness has been reported to be an important risk factor in the progression of ocular hypertension to glaucoma, as well as being frequently associated with the initial diagnosis of glaucoma (Leske et al., 2008, Gordon et al., 2002, Dueker et al., 2007). The evidence for this is discussed below.

1.3.3.3.2.2 CORNEAL THICKNESS AS A RISK FACTOR FOR THE PRGORESSION OF OCULAR HYPERTENSION TO GLAUCOMA

The first major study to demonstrate the importance of central corneal thickness as a risk factor for the progression of ocular hypertension to glaucoma was the Ocular Hypertension Treatment Study (OHTS). OHTs was a landmark multi-centre randomized controlled clinical trial. The primary aim of this trial was to evaluate the safety and efficacy of topical ocular hypotensive medication in preventing or delaying the onset of glaucomatous optic neuropathy or visual field loss in subjects with ocular

hypertension at a moderate risk for developing POAG. Its secondary aim was to identify risk factors to predict which subjects with ocular hypertension were most likely to develop glaucoma. Participants with thin corneas (555μm or less) had a three-fold increased risk of developing POAG compared with those who had a corneal thickness of more than 588μm. Though cross sectional studies had previously documented (Alsbirk, 1978, Argus, 1995, Herndon et al., 1997, Herman et al., 2001, Emara et al., 1999, Morad et al., 1998, Wolfs et al., 1997) an association between central corneal thickness and the diagnosis of ocular hypertension, NTG and POAG, OHTs was the first prospective study to demonstrate that thinner central corneas are a risk factor for the progression of ocular hypertension to POAG. Since then, this finding has been supported by others (Medeiros et al., 2003a, Medeiros*** et al., 2003, Medeiros et al., 2003b, Medeiros et al., 2005, Leske et al., 2008). It is now widely accepted that in best clinical practice, the assessment of risk of a glaucoma suspect should include the measurement of CCT (European_Glaucoma_Society_II, 2003, Dueker et al., 2007, American_Academy_of_Ophthalmology, 2008, Behki et al., 2007).

1.3.3.3.2.3 CORNEAL THICKNESS AS A RISK FACTOR FOR THE PRESENCE GLAUCOMA

The evidence for central corneal thickness as a risk factor the presence of glaucoma is more equivocal. A number of studies, including population based studies in Barbados and Rotterdam, have found a thinner CCT to be associated with the occurrence of

glaucoma (Bechman et al., 2000, Nemesure et al., 2003, Wolfs et al., 1997). Furthermore, a thinner central cornea has been associated with increased glaucomatous visual field loss as well as increased optic nerve head damage (Congdon et al., 2006, Herndon et al., 2004, Jonas et al., 2005, Kniestedt et al., 2006., Sullivan Mee et al.,2005, Sullivan-Mee et al., 2006a, Sullivan-Mee et al., 2006b). These findings are not supported by all studies (Argus, 1995, Foster et al., 2003, Herndon et al., 1997,Iwase et. al., Vijaya et. Al., 2005). There is little evidence so far to suggest that a thinner central corneal is a risk factor for the progression of glaucoma (Congdon et.al., 2006, Jonas et.al., 2005, Kim and Chen 2004, Leske et. al., 2003, Stewart et.al., 2006, Weizer et al., 2004).

1.3.3.3.2.4 CORNEAL THICKNESS IN OCULAR HYPERTENSION AND GLAUCOMA

Two main theories have arisen to explain the possible role of central corneal thickness in ocular hypertension and glaucoma. Though multivariate analysis in the Ocular Hypertension Treatment Study had demonstrated that the effect of CCT on the progression of ocular hypertension to glaucoma was independent of IOP, the first theory centers around CCT's role in the measurement of IOP. The second theory postulates that corneal thickness may be a surrogate for some yet unknown biological factor, possibly reflecting a biomechanical aspect of the optic nerve head.

1.3.3.3.2.4.1 CORNEAL THICKNESS IN APPLANATION TONOMETRY

Since it's introduction in the 1950s, the clinical gold standard for measuring IOP has been Goldman applanation tonometry (Kniestedt et al., 2008). The Goldman applanation tonometer is a variable force tonometer based on the Fick-Imbert Law- which states that "the pressure within a sphere is equal to the force needed to flatten part of the sphere divided by the area flattened" (Hart, 1992). Theoretically this law applies to perfect, dry and infinitely thin spheres. The cornea is not dry, infinitely thin nor spherical. Different factors may effect the accuracy of IOP measured by GAT – reviewed by Whitacre and Stein (Whitacre and Stein, 1993, Whitacre et al., 1993) Damji (Damji et al., 2003) and Hart (Hart, 1992) The main sources of error include the following: The tear film bathing the cornea forms a meniscus between the surface of the cornea and the applanation prism. This may lead to measurement errors in two ways. First, the meniscus may be assumed (incorrectly) to be part of the corneal contact area. Second, the surface tension of this meniscus can also effect the force required for applanation. A small volume of aqueous is displaced during applanation which leads to a small but significant increase in IOP. Finally, the flattening of the cornea will be partially dependent on its physical properties. The cornea itself has a certain rigidity (or modulus of elasticity) which resists the applanation of its surface.

GAT attempts to minimize these potential errors by maintaining only a small area of contact, so that the portion of cornea flattened and aqueous displaced is minimal. The force required to flatten a circular area of cornea with a diameter of 3.06 mm is

measured. This area was chosen in part as one at which the surface tension of the tear meniscus and the rigidity of the cornea approximately offset each other. In addition, based on the measurement of cadaver eyes, Goldman and Schmidt assumed a central corneal thickness of 500μm. It was also assumed that corneal thickness would not vary greatly in the population. They did however recognize that applanation tonometry could be influenced by the physical properties of the cornea such as thickness, curvature and modulus of elasticity, so readings could only be considered reliable if the cornea was what they deemed, "normal." (Whitacre and Stein, 1993, Chihara, 2008).

Subsequently, the importance of CCT in the measurement of IOP *in vivo*, has been demonstrated by a number of studies, starting with work by Ehlers and colleagues (Ehlers et al., 1975). Ehlers et al. performed manometry and either Perkins or Drager tonometry simultaneously on 29 eyes about to undergo intraocular surgery. A systemic error proportional to the true IOP and CCT when IOP was measured by applanation tonometry was found. Ehlers et al. calculated that intraocular pressure when measured by Goldman Applanation Tonometry, is most likely to correspond to intracameral IOP when the central corneal thickness is 520μm. Similar results have been reported by others using similar methodology (Whitacre and Stein, 1993, Kohlhaas et al., 2006, Feltgen et al., 2001). There have been a variety of other reports demonstrating a positive correlation between corneal thickness and IOP, as well as increased corneal thickness in ocular hypertension and decreased corneal thickness in normal tension glaucoma (Dohadwala et al., 1998, Foster et al., 1998, Bhan et al., 2002, Eysteinsson et al., 2002, Ko et al., 2005, Li et al., 2002, Lleo et al., 2003, Doughty and Jonuscheit, 2007). The

relationship between intraocular pressure and CCT has also been the subject of a recent meta-analysis (Doughty and Zaman, 2000) and will not be reviewed here. Overall, thick corneas tend to overestimate intraocular pressure, whereas relatively thin corneas underestimate intraocular pressure (Doughty and Zaman, 2000). Different algorithms/correction factors have been proposed to reduce the influence of CCT on IOP but none have gained universal acceptance (Dueker et al., 2007).

1.3.3.3.2.4.2 CORNEAL THICKNESS AS A SUSCEPTIBILITY FACTOR

The second hypothesis suggests that central corneal thickness may be a surrogate for biomechanics of the optic nerve head or some other yet unidentified biological factor. The Ocular Hypertension Treatment Trial found CCT was a powerful predictor of glaucoma in both univariate and multivariate analysis, suggesting that the effect of CCT on the progression of ocular hypertension may be independent of IOP (Gordon et al., 2002). This finding has been independently confirmed in the more recent European Glaucoma Prevention Study (Miglior et al., 2007a). Hence underestimation of intra-ocular pressure by Goldman applanation tonometry associated with a thinner CCT, may only partly explain the relationship between thin corneas and increased glaucoma risk. Another possibility that has been postulated to explain this association is that perhaps, CCT (and other biomechanical or physical properties of the cornea such as hysteresis) is a surrogate index for an inherent biomechanical (or other) property of the eye such as the thickness of the peripapillary sclera or degree of bowing of the lamina cribrosa.

Considering the cornea is an extension of the sclera and lamina cribrosa as is related to it by anatomy, physiology and development, this is not a far fetched hypothesis. However, though more evidence is beginning to emerge, there is little information to date about CCT as a biological risk factors or its association with optic nerve head parameters. Studying these possible associations are problematic. With current technology there is no easy way to measure the thickness of the lamina cribrosa or surrounding sclera of the optic nerve head in vivo. Most of the studies published so far are also limited by small numbers and a lack of genetic homogeneity, even though it is now well established that many of the quantitative traits related to glaucoma, including CCT show inter-population variation and are highly heritable (Toh et al., 2005).

Mark Lesk and colleagues attempted to circumvent the problems of measurement by using cup depth assessed by scanning laser tomography as a surrogate for the position of the lamina cribrosa to investigate the relationship between corneal thickness and lamina cribrosa compliance (Lesk et al., 2006). Patients with thin corneas with ocular hypertension or open angle glaucoma had greater movement (compliance) of their lamina cribrosa, as well as a smaller improvement in optic nerve head blood flow after IOP reduction than those with thicker corneas. Leske et al. hypothesized that a thin cornea may be connected to a thin sclera which in turn may reflect a thin lamina cribrosa. A thin lamina would be more vulnerable to movement induced by fluctuations in IOP – either physiological due to diurnal variation, or iatrogenic due to glaucoma therapy. Movement of the lamina may lead to the damage of RGC axons, associated vasculature and supporting cells as previously described. Nicoela et al. (Nicolela et al.,

2006) however, did not find any relationship between central corneal thickness and optic nerve parameters following IOP modulation.

As the thickness of the lamina and peripapillary sclera is difficult to measure in vivo, studies have sought to define the relationship between the thickness of the anterior sclera and CCT to find indirect evidence for an association between lamina cribrosa and posterior sclera with CCT. CCT has been correlated to scleral thickness in some studies (Albekioni et al., 2003, Oliveira et al., 2004, Oliveira et al., 2006) but not others (Oliveira et al., 2006).

The ultra structure of the posterior sclera, its collagen content and extra-cellular matrix in animal models of myopia, confers different biomechanical properties compared to emmetropic eyes (McBrien and Gentle, 2003). The lamina cribrosa of highly myopic eyes is also significantly thinner than non myopic eyes (Jonas et al., 2004). Myopia is also an independent risk factor for glaucoma (discussed in the section below). Hence others have attempted to investigated if there is a correlation between refractive error and/or axial length and CCT (Oliveira et al., 2006). In a retrospective cross sectional study of ocular parameters from patients from an ophthalmology clinic in New York, Shimmiyo et al (Shimmyo and Orloff, 2005) found no correlation between CCT and axial length. Both CCT and refractive error show average differences between populations, hence it follows that the relationship between these variables may also show such differences. Subgroup analysis of self reported ethnicity, broadly described as being of “Hispanic”, “ Asian” “Caucasian “ or “African American” did not demonstrate

any correlation. (Shimmyo and Orloff, 2005). In a similar study by Oliveira et al (Oliveira et al., 2006), 140 patients from a New York Ophthalmology clinic of varying self reported ethnicity were investigated. CCT was not found to correlate to either refractive error or axial length. It did however, correlate with scleral thickness but only at the scleral spur.

Jonas et al investigated the relationship between CCT and the thickness of lamina cribrosa directly by the examination of 111 enucleated non-glaucomatous eyes (Jonas and Holbach, 2005). The thickness of the central and peripheral lamina cribrosa and peripapillary sclera were all found to be statistically independent of mean central corneal thickness. The authors admitted that histological artifact introduced during preparation could account for this lack of association.

There is little information about the association of CCT to other optic nerve head or glaucoma associated parameters, and the evidence to date is conflicting. Soans et al (Soans et al., 2004) examined the relationship between CCT and asymmetric disc cupping in the eyes of 41 patients with glaucoma. The eyes with the greater glaucomatous cupping had a thinner cornea compared to the fellow eye. This difference was statistically significant. In a study of 51 patients with ocular hypertension and 35 normal subjects, CCT correlated significantly with RNFL measurements and with only four of the ONH parameters investigated (cup to disc area ratio, cup area, rim area and horizontally integrated rim width). Ocular hypertensive patients with a central corneal thickness of less than 555μm had significantly thinner RNFLs compared to ocular

hypertensive with thicker corneas and normal subjects with thick corneas. However, in a similar study of 109 healthy volunteers who were part of the Advanced Imaging in Glaucoma Study, CCT was not associated with retinal nerve fiber layer thickness. In a study by Iester et al (Iester and Mermoud, 2001), CCT not found to be correlated to RNFL. Henderson et al (Henderson et al., 2005) found a correlation between retinal nerve fibre layer thickness and corneal thickness in patients with ocular hypertension. Ocular hypertensives with thinner corneas had a thinner retinal nerve fiber layer. In a more recent study by Pakravan et al (Pakravan et al., 2007) of 137 patients of 9 different self reported ancestry, an inverse correlation between CCT and optic disc area was found. The authors concluded that as well as overestimating IOP, a thicker central cornea may also be indicative of a smaller (and hence more robust) optic nerve head, the converse being true for individuals with a thinner central cornea. The Ocular Hypertension Treatment Study (Budenz et al., 2006) found thinner central corneas were one of the baseline risk factors associated with associated the development of optic disc hemorrhages, a known risk factor for GON (Uhler and Piltz-Seymour, 2008). Others have not found an association between the two (Jonas et al., 2005a).

From these conflicting reports, it is apparent that the role of central corneal thickness in glaucoma onset and progression remained to be clarified, with very limited information available about the relationship between CCT and optic nerve head parameters. At the time this project was being established, there were no published population based studies which had investigated the relationship between central corneal thickness and optic nerve head parameters. Such information would be valuable in attempting to

clarify if there is a connection between central corneal thickness and biomechanical or other parameters of the optic nerve head.

1.3.3.3.3 CUP TO DISC RATIO

Axons of retinal ganglion cells, the retinal nerve fibre layer, converge to form the optic disc and subsequently the optic nerve. The convergence of the axons forms a central depression in the disc known as the optic cup. Since the introduction of the ophthalmoscope by Helmotz in the mid 1800s, certain optic nerve changes have been associated with glaucoma. The section above has discussed the possible role of optic nerve head susceptibility in the pathogenesis of glaucomatous optic neuropathy at length. There is also consistent and strong evidence that increased cup-to-disk ratio (of ≥ 0.5) is an independent risk factor for the progression of ocular hypertension and glaucoma (Friedman et al., 2004). Though progressive thinning of the neuroretinal rim or enlargement of the cup is considered the quintessence of advancing glaucoma, the Ocular Hypertension Treatment Study showed that relatively larger ratios preceded the diagnosis of glaucoma as well. However, several factors make it difficult to establish if disc size is a truly independent risk factor in glaucoma susceptibility (Friedman et al., 2004, Gordon et al., 2002, Klein et al., 2004b). First, it could be argued the increased cup-disc ratios in these patients may be an indication of early structural damage and not a risk factor. Furthermore disc parameters show great inter and intra-population

variation but comparison between studies are hindered by the difference techniques used to measure and monitor disc variables.

1.3.3.3.4 MYOPIA

Cross-sectional and case control studies, from across the continents have also found associations between glaucoma and myopia (Daubs and Crick, 1981, Ponte et al., 1994, Mitchell et al., 1999, Grodum et al., 2001, Yoshida et al., 2001, Ramakrishnan et al., 2003, Wong et al., 2003, Xu et al., 2007b). Analogous to studies in glaucoma, studies on refractive error are difficult to compare as definitions for myopia and hyperopia depend on demarcating specific values, but there is no consensus for these definitions either. Problems associated with studies investigating both glaucoma and myopia are further compounded by a lack of consensus definition or diagnostic criteria for both diseases. Not all studies however have supported the association between glaucoma and myopia. For example. In the follow up of 647 ocular hypertensive subjects, Quigley et al did not find myopia to be a risk factor for the development of glaucomatous visual field loss in ocular hypertension (Quigley et al., 1994). The results of randomized clinical trials have also been inconsistent (Gordon et al., 2002, 2002, Leske et al., 2003).

The exact biological reason for the association between myopia and glaucoma is unclear. There is evidence that the increased risk (at least in myopia $\geq$ 4D) is not due to an increased association with raised IOP (Chihara et al., 1997) but is an independent risk

factors for glaucoma. This association may be related to the structure of the myopic eye which differs from emmetropic eyes (Fong et al., 1990, Saw et al., 2005, Jonas et al., 2004). Myopic eyes have longer axial lengths and deeper anterior chambers. The lamina cribrosa is thinner and shows greater deformability in myopia than in emmetropia (Jonas et al., 2004). This greater compliance will make the ONH more vulnerable to fluctuations in IOP and subsequent RGC damage.

1.3.3.3.5 OTHER OCULAR FACTORS

Pigment dispersion and exfoliation syndrome are both associated with open angle glaucoma (Sowka, 2004a, Sowka, 2004b,Boland and Quigley, 2007), though strictly speaking, not with primary open angle glaucoma as the term primary suggests, with this form, the cause of the optic neuropathy is unknown. Other putative risk factors include optic disc haemorrhages and peripapillary atrophy (Primrose, 1970, Wilensky and Kolker, 1976, Kitazawa et al., 1986, Nevarez et al., 1988, Buus and Anderson, 1989, Jonas et al., 1992, Jonas and Xu, 1993, Jonas et al., 1989, Jonas and Naumann, 1989, Araie et al., 1994, Jonas and Iester, 1995, Sugiyama et al., 1997,Jonas and Budde, 2000, Lee et al., 2002, Yamamoto et al., 2004, Xu et al., 2007c) which were discussed in the sections before and choroidal thickness (Yin et al., 1997).

1.3.3.4 ENVIRONMENTAL RISK FACTORS

There has been no environmental risk factor consistently associated with the onset or progression of POAG (Pasquale and Kang, 2009). Even smoking, one of the most common environmental risk factors associated with a large number of chronic diseases including the acceleration of ocular conditions such as macular degeneration and cataract (Solberg et al., 1998) has not been associated consistently with the onset or progression of POAG (Edwards et al., 2008). There is some evidence that exercise can reduce intraocular pressure (Qureshi, 1996).

1.3.3.5 FAMILY HISTORY / GENETICS AS A RISK FACTOR FOR PRIMARY OPEN ANGLE GLAUCOMA

Family history is now recognized as an important risk factor for the development of POAG. Cross sectional epidemiological studies have demonstrated that 10 to 50% of POAG patients have a reported family history of the disease (McNaught et al 2000) though nearly a third of cases may be under-reported. The risk for developing the disease amongst first degree relatives is 2 to 10 fold (Tielsch 1994, Wensor 1998, Wolfs 1998). Furthermore, several large population based studies have demonstrated that the prevalence of glaucoma varies between populations of self reported ethnicity (summarized in table 1.3). A recent meta-analysis (Rudnicka 2006) calculated the average estimated prevalence of POAG in those over 70 years of age was 3% in Asian

populations, 6% in white Caucasian populations and 16% in populations of black African and Caribbean origin (table 1.6). The clinical course of POAG also differs in these populations. POAG for example, shows more rapid and early progression in blacks than white Caucasians, with the risk of irreversible blindness also being greater amongst the former (reviewed by Racette 2003, Wadhwa 2005). The predominant *type* of glaucoma also varies across ethnic groups, with population based studies suggesting that primary angle closure glaucoma predominates amongst East Asians and Eskimos whilst POAG is the more dominant form found in white Caucasians and those of Afro-Caribbean origin (Congdon 1992, He 2006).

The genetic basis of glaucoma is further supported by twin studies (Gottfresdottir 1999, Teikari 1987 and 1992). A prospective study by Gottfresdottir in 1999, for example, looking at 50 monozygotic twin pairs and their spouses, found concordance rates of open angle glaucoma in twin pairs (98%) exceeded that of spouse-twin pairs (72%). Since then, several genetic loci associated with POAG have been described and a number of genes implicated and are summarized in the table below.

TABLE 1.7: GENETIC LOCI ASSOCIATED WITH OPEN ANGLE GLAUCOMA

YEAR	LOCUS NAME	CHROMOSOMAL LOCATION	OMIM	GENE IDENTIFIED	REFERENCE STUDY
1993	GLC1A	1q21-q31	137750	MYOC	(Sheffield et al., 1993)
1996	GLC1B	2cen-q13	606689	-	(Stoilova et al., 1996)
1997	GLC1C	3q21-q24	601683	-	(Wirtz et al., 1997)
1998	GLC1D	8q23	602429	-	(Trifan et al., 1998)
1998	GLC1E	10p15-p14	137760	OPTN	(Sarfarazi et al., 1998)
1999	GLC1F	7q35-q36	603383	-	(Wirtz et al., 1999)
2004	GLC1J	9q22	608695	-	(Wiggs et al., 2004)
2004	GLC1K	20p12	608696	-	(Wiggs et al., 2004)
2005	GLC1G	5q22	609887	WDR36	(Monemi et al., 2005)
2005	GLC1I	15q11-q13	609745	-	(Allingham et al., 2005)

2005	GLC1L	3p21-22	137750	-	(Baird et al., 2005a)
2006	GLC1M	5q22.1-q32	601535	-	(Pang et al., 2006)
2006	GLC1N	15q22-q24	611274	-	(Wang et al., 2006b)
2007	GLC1H	2p15-p16	-	-	(Suriyapperuma et al., 2007)

The first locus for POAG, mapped to chromosome 1 band q21-q31 locus, was identified by linkage analysis in a 37 family member pedigree affected with an autosomal dominant form of juvenile open angle glaucoma (Sheffield et al., 1993). The locus was named GLC1A – GLC for glaucoma, 1 for 'primary open angle' and A for the first linkage site. The finding was confirmed in subsequent linkage studies in both JOAG and POAG and the gene, known as myocilin (MYOC) or the TIGR gene, identified. The association between myocilin and POAG was confirmed by Stone et al (Stone et al., 1997)who screened 330 unrelated POAG patients and 471 controls. *MYOC* mutations were found in 3.9% of POAG patients but only 0.2% of controls ($p<0.05$). Subsequent analysis of *MYOC* mutations have shown their frequency to be between 2 to 4% in glaucoma patients (Fingert et al., 1999). These mutations are found in patients from a variety of ethnic backgrounds. Sequence analysis of the myocilin gene suggests protein in the region of 55kDa (reviewed elsewhere – see Johnson (Johnson, 2000) and Libby

(Libby et al., 2005) widely expressed in both ocular (the trabecular meshwork, the retina and the ciliary body) and extraocular tissues (Fingert et al., 1998). In vitro, myocilin has been observed to modify fibroblast dispersal (Peters et al., 2005), be upregulated in the central nervous system response to injury and hinder the development of neuritis (Jurynec et al., 2003), over-expression/extracellular myocilin has been observed to effect the function of mitochondria as well as reduce the adhesive properties of trabecular meshwork cells (Wentz-Hunter et al., 2004a, Wentz-Hunter et al., 2004b, Sakai et al., 2007). MYOC's alternate name of TIGR (trabecular meshwork inducible glucocorticoid response protein) arose as it was identified as being upregulated in trabecular meshwork cells that had been treated with dexamethasone (Nguyen et al., 1998). Despite these observations, myoclin's normal physiological function in vivo remains unclear. Over 70 disease causing myocilin mutations have been identified and there is some evidence to suggest that method by which mutant myocilin facilitates glaucomatous optic neuropathy is through it's aggregation in trabecular meshwork cells, causing increased resistance to aqueous egress and subsequently increased IOP (Nguyen et al., 1998, Lutjen-Drecoll et al., 1998, Fautsch et al., 2000, Clark et al., 2001, Fautsch et al., 2006, Naskar and Thanos, 2006). This theory is not supported by all studies (Caballero et al., 2000, Gould et al., 2004, Zillig et al., 2005).

The second POAG gene identified was optineurin (optic neuropathy inducing protein), *OPTN*, initially identified by linkage analysis of a large British pedigree in which 15 of 46 members had normal tension glaucoma (Sarfarazi et al., 1998). The GL1E locus was mapped to 10p15-14. Known to be expressed in the retina and to have a role in

apoptosis, OPTN was identified as a good candidate. Individuals with specific OPTN mutations are known to have a more relentless form of NTG but on the whole, OPTN variants are a rare cause of normal tension glaucoma. OPTN is known to interact directly with several different proteins involved in cellular morphogenesis and membrane trafficking (see Sarfarazi and Rezaie 2003 (Sarfarazi and Rezaie, 2003) for a comprehensive review). The exact mechanism by which OPTN contributes to the glaucoma phenotype remains to be elucidated.

A third gene WDR36, was identified as a possible candidate following the discovery of the GLC1F locus by linkage analysis (Monemi et al., 2005). A role in glaucoma pathogenesis via T cell activation has been postulated for its gene product. The WDR36 D658G mutation was present in a large GLC1G-linked family and though other disease susceptibility mutations were described, this variant was the only one which was statistically significant in cases compared to controls. This has not been replicated elsewhere (Hewitt et al., 2006) and a recent analysis of WDR36 sequence variants did not show consistent segregation with POAG afflicted individuals, though certain variants were found more frequently in patients with more severe disease, suggesting that the role of WDR36 may be as a glaucoma modifier rather than a causative gene (Hauser et al., 2006).

Over 20 other gene variants associated with POAG have been reported from association studies (Copin et al., 2002, Fujiwara et al., 2003, Funayama et al., 2006, Inagaki et al., 2006, Ishikawa et al., 2005, Junemann et al., 2005, Juronen et al., 2000, Lam et al.,

2006, Lin et al., 2002, Lin et al., 2004, Lin et al., 2003a, Lin et al., 2003b, Lin et al., 2006, Liu et al., 2007, Logan et al., 2005, Mabuchi et al., 2005, Melki et al., 2004, Shibuya et al., 2008, Tosaka et al., 2007, Tsai et al., 2003, Tsai et al., 2004, Tunny et al., 1998, Tunny et al., 1996, Unal et al., 2007, Vickers et al., 2002, Pang et al., 2006,Wang et al., 2006a). Most have been reported in single studies and not always replicated in other. The actual role of these genes in POAG remains to be clarified (Hewitt et al., 2006).

Mutations in known POAG genes and classic Mendelian inheritance accounts for only a minority of POAG patients (Fan et al., 2006). More and more evidence suggests that in the majority of cases, POAG is not a single gene disorder but a complex trait (Fan et al., 2006, Hewitt et al., 2006). Like other diseases of late onset, the susceptibility (or liability) to POAG can be considered to be normally distributed and the net result of genetic and environmental influences (Wright et al., 2003). For POAG to be manifest, a certain threshold of liability must be surpassed. This high level of complexity has added an extra degree of difficulty in identifying associated susceptibility genes.

1.4 DIAGNOSIS

The diagnosis of POAG is made primarily by the presence of glaucomatous neuropathy (cupping) and a compatible visual field defect in the presence of an open, normal anterior chamber angle, without the presence of other ocular abnormalities which may

contribute to these changes. (European_Glaucoma_Society, 2003). Raised intra-ocular pressure used to be included as part of the definition, but population based studies have consistently confirmed that many patients with glaucoma have an IOP below 21mm-Hg. (Hollows and Graham, 1966, Iwase et al., 2004, Klein et al., 1992b, Sommer et al., 1991). IOP now is considered to be a risk or contributing factor rather than the sole causative factor. However, over the years, there has been little consensus on the criteria used for the diagnosis of glaucoma and no consensus on the methods and standards used to define optic disc damage or visual defects. Criteria for diagnosis has varied from using visual field and optic disc criteria, to visual field or optic disc criteria alone, to disc changes and IOP criteria to intraocular pressure alone. For example, in a review of 182 articles published between 1980 and 1995, by Bathija and Gupta et al. in 1998, only 66% of articles included in the analysis included a definition for open angle glaucoma. Of these articles, 36% used both optic disc and visual field changes, 13% used optic disc or visual field changes, 26% used only visual field criteria, 20% used only IOP and 5% used only optic disc criteria. The definition for normal tension glaucoma, a subset of POAG, and ocular hypertension has also been found to be highly variable (Tavares et al., 2006, Lee et al., 1998). A lack of a clear gold standard definition for glaucoma, can lead to a false dichotomy between detection of damage (going from disease to no disease) and disease progression (going from mild to more severe disease). This variability in definition, classification, and criteria used for diagnosis has made comparison and summation of results from glaucoma related studies problematic.

1.5 MANAGEMENT

The goal of glaucoma management as described by the European Glaucoma Association and the American Academy of Ophthalmology as well as a number of other care guidelines, is to maintain a patient's quality of life by preserving visual function at, as stated by the EGA " at a sustainable cost" (European_Glaucoma_Society, 2003) The cost described is not merely a financial one to the individual or society but also one in terms of treatment side effects and inconvenience. The mainstay of POAG treatment remains the lowering of intraocular pressure either by means of medication, surgery or laser and over the past few years a number of multicentre trials have confirmed the merits of lowering IOP in primary open angle glaucoma as well as ocular hypertension (Gordon et al., 2002, Kass et al., 2002, Anon, 1998b, Anon, 1998a, Heijl et al., 2002, Lichter et al., 2001). Management involves lowering an individuals IOP to a certain "target IOP", and then maintaining IOP at that level (European_Glaucoma_Society, 2003). The target IOP for an individual or eye is the level of IOP in that individual or eye, at which further glaucoma optic neuropathy will not occur. Methods for calculating target IOP have been described but in practice, the individual response to IOP is so variable, this is difficult to establish accurately for each individual and eye. Other putative treatments, such as neuro-protective agents, have been suggested as well but their role in POAG management remains to be clarified (Weinreb, 2007).

1.6 PROGNOSIS

Despite a number of large studies demonstrating the value of lowering IOP in POAG and OHTN, for a number of individuals this does not arrest or slow the progression of glaucoma (Kass et al., 2002, Anon, 1998b, Heijl et al., 2002, Lichter et al., 2001). This suggests that there may be other factors, not just IOP important in the progression of glaucomatous optic neuropathy in these individuals.

1.7. THE FUTURE OF GLAUCOMA MANAGEMENT

The fact that is relentlessly highlighted then re-emphasized about primary open angle glaucoma is the heterogeneous nature of the disease. Unfortunately, in the case of glaucoma, one size does not fit all and it is only by truly understanding the details of its aetiology and pathogenesis will we be able to diagnose, treat, monitor, and hopefully, one day prevent the disease. The behavior of primary open angle glaucoma shows great inter-individual as well as inter-population variation. Ideally, based on our knowledge of the genetic and environmental factors that may influence the disease, and the cascade of events which eventually lead to its expression in each individual, we would be able to assess an individual's susceptibility to disease and provide personalized programs for disease prevention, treatment, monitoring and prognosis. The treatments we use would be tailored to suit each individual not just based on our comprehensive understanding of disease pathogenesis but also taking into account individual responses to drugs.

However, for this dream of personalized medicine to become a reality, we need a better understanding of the genetic and environmental factors that may influence primary open angle glaucoma susceptibility.

The primary goal of this PhD is to establish a study investigating the inheritance of quantitative traits related to primary open angle glaucoma in the Scottish population isolate of Orkney. The study will be designed to be a long term project to collect quantitative trait data for a quantitative trait analysis to continue after the conclusion of this PhD. The ultimate aim of this study is to identify new genes associated with a predisposition to POAG and to locate regions of the genome influencing disease risk. Chapter 2 summarizes and describes the aims, objectives and research questions for this PhD thesis. Chapter 3 gives an overview of the project and our rationale for using this particular approach and a description of setting up the initial project. Chapter 4 continues with this theme, describing the handling and analysis of data. Chapter 5 is an overview of the results followed by further results and discussion in chapters 6 to 8. Chapter 9 brings these results together in a final discussion.

1.8. SUMMARY

Primary open angle glaucoma is a progressive optic neuropathy which is the primary cause of irreversible blindness in the world. Treatment involves lowering intra-ocular pressure but for a number of patients this does not abrogate or slow disease progression.

Many risk factors have been associated with the disease but only age, ancestry, family history, intraocular pressure, central corneal thickness and optic nerve head parameters have been associated relatively consistently across studies. The aetiology and pathogenesis of POAG is poorly understood but available evidence suggests it is multi-factorial. Genetically, POAG can be considered a complex disease where both genetic and environmental factors act in concert to cause the disorder. Though several genes and loci have been identified, they appear to be responsible for only a small number of POAG cases. Hence, a considerable amount of work is required before the aetiology and pathogenesis of primary open angle glaucoma is fully understood.

CHAPTER 2

AIMS AND OBJECTIVES

1.1 AIMS AND OBJECTIVES

1.1.1 The primary objective of this PhD project was to design and establish a study investigating the inheritance of quantitative traits associated with primary open angle glaucoma in the Scottish population isolate of Orkney. The study was designed to be a long term project to collect quantitative trait data for a genetic analysis to continue after the conclusion of this PhD. The final aim of the Orcades Eye Study is to identify new genes associated with a predisposition to POAG and to locate regions of the genome influencing disease risk. During the time frame of this PhD, funding was sourced, premises found and infrastructure built, ethical approval finalized, volunteers recruited, tools and protocols for data collection, entry and storage trialled and confirmed. Also data on over 10 quantitative traits associated with POAG was collected. These traits included entral corneal thickness (CCT), intraocular pressure (IOP) and optic nerve head (ONH) parameters such as rim area, cup area, cup volume, cup depth and peripapillary atrophy. Refractive error and axial length were also measured as myopia is a risk

factor for POAG. Chapter 3 discusses in depth the reasons for this particular choice of population and study design, describes how funding was sourced, equipment and premises chosen and discusses the methods used for phenotyping. Chapter 4 will discuss data management and analysis.

1.1.2 The secondary aim of this PhD was to describe the findings from this initial sample of data collected during this time period. We analysed the distribution and preliminary statistics of the ocular quantitative traits of central corneal thickness, intra-ocular pressure and optic nerve head parameters as these are well established risk factors for primary open angle glaucoma (Leske and Rosenthal, 1979, Leske, 1983, Boland and Quigley, 2007). There is evidence that quantitative traits such as IOP, CCT and ONH parameters show variation between populations (Katavisto and Sammalkivi, 1964, Armaly, 1965, Hollows and Graham, 1966, Wallace and Lovell, 1969, Alsbirk, 1970, Kahn et al., 1977a, Kahn et al., 1977b, Shiose and Kawase, 1986, Shiose, 1990, Sommer et al., 1991, Shiose et al., 1991, Klein et al., 1992a, Dielemans et al., 1994, Mitchell et al., 1996, Leske et al., 1997, Jacob et al., 1998, Bonomi et al., 1998, Weih et al., 2001, Doughty and Zaman, 2000, Rotchford and Johnson, 2002, Racette et al., 2003, Hashemi et al., 2005, Xu et al., 2005, Rudnicka et al., 2006). However there is very little information regarding the distribution of quantitative traits associated with glaucoma from a Scottish population, let alone Orkney. Hence the analysis of these traits will allowed us to investigate the mean values and normative

ranges for this population in addition to providing information about possible genetic architecture from phenotypic data.

Despite central corneal thickness being one of the most powerful predictors for the progression of ocular hypertension to glaucoma, and despite the increasing interest in the cornea as a possible surrogate for some factor reflecting the optic nerve head or optic nerve head biomechanics, there is little information regarding the relationship between corneal thickness to other optic nerve head or refractive parameters. The evidence that is available is often conflicting and derived from genetically heterogeneous populations of self reported ancestry (Soans et al., 2004, Pakravan et al., 2007). When glaucoma exhibits such genetic heterogeneity, it follows that the relationships between its associated quantitative traits may also show inter-population variation. The archipelago of Orkney has been geographically isolatedfor many centuries, with high levels of endogamy and a reduced population size which has lead to reduced genetic diversity in the archipelago compared to more admixed populations found, for example, in some ophthalmology clinics of the United States sometimes used in these studies (McQuillan, 2009, McQuillan et al., 2008). We also therefore investigated the relationship between the quantitative traits related to glaucoma in this study sample. Few published studies have investigated the relationship between quantitative traits from such a wide range of parameters, as we plan to gather.

2.1.3 Finally we had planned to investigate the heritabilities of these traits, followed by a definitive genetic analysis using association and linkage methods to identify genes and regions of the genome asscociated with glaucoma related quantitative traits .

1.2 RESEARCH QUESTIONS

1.2.1 Population based studies from across the world have demonstrated that quantitative traits associated with primary open angle glaucoma such as intraocular pressure and optic nerve head parameters can vary between populations. What are the distributions of the quantitative traits associated with primary open angle glaucoma from the population isolate of Orkney ? In particular what are the distributions of central corneal thickness, intra-ocular pressure and optic nerve parameters ? Chapter 4, sections 4.1.2 to 4.1.6 inclusive describes the measurement of these traits, chapters 6-8, the results, and in figures 6.1, 7.1, 8.1-5 demonstrate the distribution of CCT, IOP and optic nerve head parameters respectively.

1.2.2 The genetic and environmental factors which influence these traits amongst the Orkney population are also different compared to other populations. How do these trait distribution and values compare to other published populations? In the results chapters 6-8, table 6.5, 7.3 and 8.2 compare Orcades results

with other published populations for the traits of CCT, IOP and optic nerve head parameters respectively.

1.2.3 Research analyzing the relationship between these traits have highlighted important associations between traits, such as the correlation between intraocular pressure and central corneal thickness (Doughty and Zaman, 2000). However, these findings have not been consistent across populations. Are there any relationships between the quantitative traits measured in our study and do they reflect the findings of other groups? Overall, chapter 4, sections 4.1.2 to 4.16 describe the measurement of these quantitative traits, and , section 4.3.3 discusses the multivariable analysis, and tables 6.2, 6.4 and 7.2 summarizes the results with further discussion within the text of chapters 6 to 8. The relationships described belwo are of particular interest.

1.2.3.1 It has been suggested that the thickness of the cornea may be a surrogate for some yet undescribed biomechanical factor of the optic nerve head and surrounding sclera (Brandt, 2007). This research has been hampered by the difficulty of studying the optic nerve head in vivo. In our study, we will collect data on a multitude of optic nerve parameters including optic disc area, optic cup area and optic cup depth (as a surrogate for lamina cribrosa compliance) and peripapillary atrophy which from the evidence provided by biomechanical models of the optic nerve head we postulate will increase the susceptibility of the optic nerve head to IOP related stress and strain and has

already been described as a risk factor for open angle glaucoma (Primrose, 1970, Wilensky and Kolker, 1976, Nevarez et al., 1988, Buus and Anderson, 1989, Jonas et al., 1992, Jonas and Xu, 1993, Jonas et al., 1989, Jonas and Naumann, 1989, Araie et al., 1994, Lee et al., 2002, Bellezza et al., 2000, Burgoyne et al., 2005, Xu et al., 2007c). Is there a relationship between central corneal thickness and optic nerve head parameters? We postulate that a thin central cornea which is associated with an increased risk of primary open angle glaucoma will be associated with factors that will increase the vulnerability of the optic nerve head such as an increased lamina cribrosa compliance and increased peripapillary atrophy. Section 4.1.6 describes the measurement of CCT, section 4.1.5 details the analysis of optic nerve head parameters, section 4.3.3 describes the multivariable analysis, and table 6.2 summarizes the results with further discussion sections 6.2 and 8.2.

1.2.3.2 In addition, myopia is an independent risk factor for glaucoma and the ultra structure of the posterior pole in animals models of myopia confers different biomechanical properties compared to emmetropic eyes (Daubs and Crick, 1981, Ponte et al., 1994, Mitchell et al., 1999, Grodum et al., 2001, Yoshida et al., 2001, Jonas et al., 2003a, Ramakrishnan et al., 2003, Wong et al., 2003, Shimmyo and Orloff, 2005, Oliveira et al., 2006, Xu et al., 2007b). Hence is there any relationship between refractive error/axial length, as a surrogate for scleral structure with central corneal thickness. We postulate that corneal thickness will show a negative correlation with refractive

error/axial length as elongation of the globe may be coupled with thinning of the cornea. Section 4.1.2 describes the measurement of refractive error, 4.1.3 describes the measurement of axial length. Central corneal thickness and a description of the multivariable analysis occurs in sections 4.1.6 and 4.3.3 as previously stated, and results summarized in table 6.2 with futher discussion in section 6.2.

1.2.4 What are the heritabilities of these quantitative traits?

1.2.5 What genes and regions of the genome are associated with these traits ?

Due to a number of reasons discussed in detail in Chapter 5, the start date of the project was postponed and after the project was started, it was subject to a number of problems which lead to delays in its continuation. We were unable to reach our target sample size of 1000. Hence during the second year of the PhD, the objectives and research questions were revised and limited upto and including question 2.24. In addition, all volunteers who participated were not genotyped in time for analysis within the time frame of this PhD and only a limited number of individuals in the team had access to this genetic information. Due to limited manpower, and the lack of access to the genetic data, time constraints of the PhD, only a limited amount of analysis in a limited number of individuals could be accomplished within this time frame.

The study of quantitative traits influencing POAG has already provided evidence for possible disease mechanisms. This project will lead to an improved understanding of the underlying aetiology and pathogenesis of primary open angle glaucoma, with the potential for developing new methods of diagnosis and treatment using this knowledge, in the future.

CHAPTER 3

ESTABLISHING THE ORCADES EYE PROJECT

3.1 OVERVIEW

The following two chapters describe setting up the Orcades Eye Project. Chapter three concentrates on overall study design including the choice of equipment, population and premises, to the sourcing of funds, conversion of premises. Chapter 4 concentrates on data collection, management and methods of analysis.

3.2 DEFINING THE PHENOTYPE

Much of the research into the genetics of glaucoma has involved treating glaucoma as a binary or qualitative trait. This approach poses several problems. The first is the lack of a standard definition for the very heterogeneous disease that is buried beneath the solitary term “glaucoma”. This is demonstrated in table 1.3 which shows the differences in definition between published epidemiological studies. This is further compounded by the different methods of visual field, optic disc and IOP assessement, and criteria used to diagnose glaucomatous optic neuropathy, visual field defects and “normal” intraocular pressure. This was investigated more formally in a literature review by Bathija et al.

(Bathija et al, 1998) and in the Rotterdam study when Wolfs et al., demonstrated the importance of having a standard definition for open angle glaucoma even further by showing the prevalence of open angle glaucoma in Rotterdam could vary 12 fold (from 0.1 to 1.2%) depending on how a case of open angle glaucoma was defined, different definitions for the disease having been obtained from other major population based studies (Wolfs et al., 2000).

The second is the prevalence of POAG. The prevalence is only around 1-4% in the over 50 population of white Caucasian ancestry. Though this prevalence is sufficient for POAG to be classified as a common disease, a considerable amount of effort still needs to be expended in order to find a sufficient number of cases for a meaningful genome-wide association study, if association methods are to be used. POAG is also a disease of late onset. Hence, finding large enough pedigrees for linkage analysis is problematic. A method of circumventing some of these issues is, rather than studying the disease itself, is to dissect the disease into contributing components in the form of quantitative traits.

In general, a quantitative trait or QT, could be considered to be one whose distribution is continuous, for example blood pressure, as opposed to a discrete trait such as blood group. QTs can be subdivided into three main categories (Hartl, 1999). Metric traits include many traits of clinical importance such weight, blood pressure, intraocular pressure and axial length. These traits can take any numerical value and are measured on a continuous scale. Meristic traits consist of integers and are measured by counting. For example, egg production and bristle number. Finally, threshold traits are traits which on

the surface appear to be binary traits, but reflect an underlying continuous liability determined by the interaction of both genetic and environmental factors. Once a certain threshold is exceeded, the defined trait becomes manifest. This paradigm probably describes the pathogenesis of most common disorders including glaucoma.

The genetic basis for such a continuous distribution in accordance to Mendel's rules was expounded in the early 1900s by R.A. Fisher (Fisher, 1918, Plomin et al., 2009). Fisher showed that the continuous distribution shown by quantitative traits could be accounted for by Mendelian inheritance if multigenic inheritance was assumed. In the majority of QTs, this continuous distribution will reflect the collective action of both multiple genetic and environmental factors. Discrete variation that may be observed with the genotype frequency distribution alone is likely to be blunted by environmental factors and the discrete assemblage converted to a smooth curve. Even for traits associated with single genes, such as phenylalanine levels, a continuous phenotypic distribution is possible if there is sufficient environmental variation (Relethford, 2007). As both genetic and environmental factors are involved, QTs are often referred to as complex traits. Because of possible involvement of multiple genes sometimes the term "polygenic trait" is used as well. The genetic loci involved with these traits are known as quantitative trait loci or QTLs.

QTL mapping has been used successfully to identify disease associated loci and genes in a number of common diseases including, diabetes and body mass index (Sladek et al., 2007), schizophrenia (Moises et al., 1995) and asthma (Ober et al., 1998, Ober et al.,

2000, Laitinen et al., 2001). In turn, case-control GWAS strategies have lead to the identification of loci which influence quantitative traits such as the inflammatory response in Crohn's Disease (Plomin et al., 2009). Glaucoma is an excellent candidate for a QTL based strategy as it is readily separated into QTs that are clinically important risk factors for the disease and are also relatively easy to measure accurately. These traits include central corneal thickness (CCT), intraocular pressure (IOP), anterior chamber depth (ACD), and optic nerve head parameters such as cup disc ratio (CDR) which were discussed at length in chapter 1.

There is mounting evidence that these three risk factors, IOP, CCT and CDR have a considerable genetic component. All show inter-population variation. IOP, for example, is on average higher in those of black African descent than white Caucasians, with the rise of IOP with age being more marked in the former population (Shiose, 1990, Semes et al., 2006). Individuals of Afro-Caribbean origin also have larger optic discs and thinner cornea compared to Caucasians (Racette et al., 2003). In addition, these traits show moderate to high *heritability*.

The origins of the term heritability remains undetermined but there is evidence to suggest it has been used since the 19th century (Bell 1977). It's meaning appears to have undergone several incarnations. Initially the term was used to suggest the transmission of characteristics from one generation to another in the broadest sense around the early to mid 1800s. Its current usage arose around the early to mid 1900s and it's final incarnation credited to the geneticist Jay L. Lush (Bell, 1977). Currently the term

describes the relative contribution of hereditary in determining a particular phenotype, formally defined as the "proportion of genotypic variance relative to phenotypic variance," (Falconer and McKay 1996). Under a polygenic model, phenotype, P, can be considered as the function of genetic effects, G, coupled with environmental effects, E, expressed as P = G + E. This can also be expressed as a function of variance components ($V_P = V_G + V_E$). These components can be used to define different types of heritability. Heritability in the broad sense describes the ratio of total genotypic variance to total phenotypic variance ($h^2_B = V_G/V_P$). V_G can be further divided into additive, dominance and epistatic effects. Dominance effects arise due to the interaction of alleles at the same locus whereas epistatic effects are secondary to the interaction of alleles at different loci. Narrow sense heritability is defined as the proportion of genetic variation due to additive genetic variance ($h^2_N = V_A/V_P$). Additive effects occur when the effects of alleles at several loci combine to contribute towards the phenotypic value. Though often no distinction is made between broad and narrow sense heritability, it is additive genetic variation which is the main cause of resemblance between relatives (Hill et al., 2008). A number of study designs and statistical techniques have been developed to estimate heritability, using a variety of study designs such as twin and family based designs regression/correlation methods, variance components and maximum likelihood (Burton et al.,2005, Rice and Borecki 2001, Thomas 2004). The value of heritability lies between 0 and 1 as it is a ratio between two components. The interpretation of this value is complex, with a number of associated caveas (Burton et.al.,2005). Despite these limitations, it can guide the choice of trait and population when designing

advanced genetic studies as the power of a study to discover new genes is positively associated with it's heritability.

Early calculations of the heritability of IOP in the normal eye based on family studies ranged from 0.4 to 0.9 (Armaly, 1967, Levene et al., 1970); heritability of the size of the normal optic nerve head between 0.6 to 0.8 (Schwartz et al., 1975) and CCT between 0.6 to 0.7. Population-based studies support the theory that both IOP and cup-to-disc ratio are moderate to highly heritable traits (Klein et al., 2004a, Chang et al., 2005)- further supported by twin studies for all three QTs (Schwartz et al., 1975, Kalenak and Paydar, 1995, Toh et al., 2005). By the time this project was established, several novel loci had been described as potential linkage regions for IOP on chromosomes 2, 5, 6, 7, 10, 12, 13, 14, 15 and 19 (Charlesworth et al., 2005, Rotimi et al., 2006, Duggal et al., 2007) and for maximum vertical cup disc ratio on chromosome 1p23 (Charlesworth et al., 2005).

IOP, CDR and CCT are all readily measurable QTs, which are less complex to investigate and analyze than POAG itself. By investigating QTs associated with glaucoma rather than disease per se, the issues which plague disease definition disappear. Measurements can be taken within the general population, amongst individuals without the disease, which greatly enhances statistical power. By measuring multiple QTs it will be possible to investigate genetic overlap between QTs. By studying the QTs associated with diseases of late onset, QTs which tend to be physiological,

anatomical or biomechanical variables, again, rather than disease per se, it becomes possible to use linkage methods as well if information is collected from pedigrees.

Hence for the Orcades Eye Project, we have chosen to study the following QTs associated with POAG: CCT, IOP, ACD, and optic nerve head parameters. As well as being an ocular condition which contributes to a significant amount of visual impairment both locally and globally, and hence worthy of investigation in it's own right, different forms of refractive error are also associated with glaucoma (Wu et al., 2000, Saw et al., 2005, Greve and Furuno, 1980). The majority of components that are associated with refraction are also easily and reliably measured. So in addition to the above QTs, we will take this opportunity to gather data about axial length (AL) and corneal curvature.

3.3 STUDY POPULATION

3.3.1 CHOICE OF POPULATION

The choice of population is an important factor when establishing a study to map disease related genes (Wright et al., 1999). The term "population isolate " describes a group of potentially interbreeding individuals who have had little genetic interchange with surrounding populations over many generations (Jobling et al., 2004). The reasons for isolation may be geographical- for example the isolates of Finland and Iceland, or religious, such as Ashkenazi Jewish populations and the Hutterites, or there may be

other socio-cultural reasons such as those of the European Roma. The term "transnational isolate" is sometimes used to describe populations which may show a wide geographical dispersion, but remain genetically isolated due to the practice of endogamy, or within group marriage (Kalaydjieva et al., 2001). Isolate populations proffer certain advantages over more outbred ones in the mapping of both Mendelian and complex diseases. These features of isolates have been reviewed extensively elsewhere (Kristiansson et al., 2008, Varilo and Peltonen, 2004, Peltonen et al., 2000) and are discussed briefly below.

There are several characteristics of isolate populations that make them interesting in the context of disease gene mapping. Many isolates tend to be formed originally by a small number of individuals. This can cause a genetic phenomenon known as the "founder effect", defined by Ernst Mayr in 1963 as " the effect of establishing a new population by a few original founders…..who only carry a small fraction of the total genetic variation of the parental population." (Mayr, 1963). When a new population is established (the founder event), the population may be formed by only a limited number of individuals. The remote Atlantic archipelago of Tristan de Cunha for example, was originally founded by around 20 individuals in the early 19th century (Roberts, 1971). These original founders will only carry a subset of alleles of the parent population. Some alleles from the ancestral population will be lost and others enriched. Hence the genetic diversity of the new population will be decreased compared to the parent population (Ridley, 2004). Even if the original population was substantial, the founder effect can occur if a population passes through a "bottleneck" - that is the population diminishes in

size due to, for example, catastrophic climatic or disease related events, war or famine, leaving only a few survivors. For example, since being established in the early 19th century, Tristan da Cunha has experienced two major population bottlenecks due to accidents/emigration – the first around 1860 when the population plummeted from 103 to 33 individuals and the second around 1890 when the population decreased from 106 to 59 individuals (Roberts, 1971). There is also evidence that our lineage, as a species, may have undergone severe population bottlenecks in the past – bottlenecks which many account for some of the reduced diversity observed in humans (Hawks et al., 2000, Rampino and Ambrose, 2000).

The next phenomenon of interest which will influence allele frequencies is genetic drift. The term "genetic drift" describes the chance fluctuation in allele frequencies due to the random contribution from each individual to subsequent generations in a finite population (Jobling et al., 2004). The impact of drift depends on the allele frequency of the founders as well as the demographic characteristics of the population such as population size, growth rate and fertility distribution. Isolation may also reduce opportunities for exogamy. Limited population size will mean that there will be a high probability that any marriage that occurs in the population will be between related individuals - even if attempts are made to avoid consanguinity. Inbreeding in a population can be measured using the inbreeding coefficient which measures the probability that two alleles of an individual will be identical by descent (Hartl, 1999). This tends to be higher in isolate populations compared to more heterogeneous ones.

With successive generations, the population will undergo repeated cycles of inbreeding and genetic drift. If a population is rebounding from a recent catastrophe, it will experience further inbreeding and drift – the more prolonged the recovery, the greater opportunity drift has to affect allele frequencies (Peltonen et al., 2000). Even if the genetic variation of the original founders was representative of the parental population though reduced in frequency, drift will encourage a change in allele frequencies. Hence, one of the main consequences of genetic isolation is population subdivision, or the emergence of a population that exhibits partial genetic differentiation compared to surrounding populations. On average, members of the isolate tend to be more closely related than members of adjacent populations. However, common marker alleles and common haplotypes are less likely to perish completely – unless the original number of founders was miniscule (Peltonen et al., 2000). Finland for example, has for many centuries remained somewhat isolated from it's European neighbors geographically but even more so culturally as it's main language, Finnish, a language of Finno-Ugric descent, whose closest linguistic brethren is Magyar and Estonian, is unintelligible to the majority of its neighbors (Kere, 2001). The genetic isolation of Finland has allowed a change in disease allele frequency within the population compared to other more heterogeneous European populations – a phenomenon labeled the "Finnish Disease Heritage." Around 40 disease phenotypes have been found to more be common in Finland than other parts of the world. Diseases of ophthalmic importance include Usher Syndrome, Cohen Syndrome, cornea plana, x-linked choroideremia and retinoschisis. However, other genetic conditions common in populations of European descent such as

cystic fibrosis, are rare in Finland. Some common alleles however, have similar frequencies to other European populations (Kere, 2001).

This reduced genetic diversity caused by an the initial founder effect and subsequently, some alleles driven to fixation and others to extinction by genetic drift, and increased levels of inbreeding has obvious advantages for the mapping of Mendelian diseases, and several isolates have been used successfully to identify genes associated with a number of conditions of ophthalmic importance. These include Cohen Syndrome (Falk et al., 2004), complete achromatopsia (Sundin et al., 2000, Rojas et al., 2002), cone rod dystrophies (Sankila et al., 2000, Jalkanen et al., 2006) Usher Syndrome (Ebermann et al., 2007, Ouyang et al., 2003, Ness et al., 2003), and retinitis pigmentosa (Koenekoop et al., 2003, van Soest et al., 1994).

Reduced genetic heterogeneity also has advantages in complex disease mapping, One of the problems that can add further background “noise”, obfuscate the association signal and hence hamper the mapping of complex disease genes is the locus and allelic heterogeneity associated with common complex diseases (Kristiansson et al., 2008). Even within an isolate, it is likely that genetic heterogeneity exists. However it is reasonable to assume that compared to more heterogeneous populations, the number of susceptibility alleles will be much reduced.

The basis for genome-wide association studies is the “common disease common variant hypothesis” which suggests that the genetic component of common diseases is partly

due to allelic variants that are relatively frequent (1-5%) in the population (Manolio et al., 2009). Despite the considerable success of GWAS in identifying a number of susceptibility alleles associated with common diseases over the past few years (Maller et al., 2006), for many common diseases only a small proportion of heritability has been identified. For example, the estimated heritability for human height is around 80% and although around 40 susceptibility loci have been identified, this only accounts for about 5% of observed phenotypic variance (Manolio et al., 2009). There are several possible explanations for this "missing heritability". The idea pertinent to this discussion is the possibility that rare variants, risk alleles found at low frequency (less than 1%) in the population, may play an important role in common disease susceptibility. The founder effect, bottlenecks and drift may enrich the frequency of certain rare alleles in population isolates, thus increasing their probability of detection in genome-wide studies (Kristiansson et al., 2008).

In addition to reduced genetic diversity, several other characteristics of isolates make them attractive study populations in complex disease mapping. In monogenic conditions, the genetic component plays the predominant role in disease aetiology. In common complex disorders, in addition to genetic factors, environmental factors are important, so reducing environmental variance will aid disease gene mapping. In isolate populations environmental influences tend to be more homogenous (Peltonen et al., 2000). Isolates often have shared religious/cultural beliefs, a more homogenous diet and lifestyle compared to more urbanized, admixed societies. Demographic records and genealogy information are sometimes more complete and accessible and it is possible to find large

extended pedigrees. For example, since it's initial founding in the early 19th century, detailed demographic records have been kept in Tristan de Cunha (Roberts, 1971). Many Nordic countries such as Iceland and Finland have extensive registries of marriages, births, migration and deaths dating back several centuries (Peltonen et al., 2000). Furthermore, levels of linkage disequilibrium are also purported to be extended over larger regions in certain "younger" isolate populations, which would be advantageous in complex disease mapping efforts (Service et al., 2006). Isolates such as Finland and Sardinia for example, require around 30% less markers to attain genome-wide coverage than more heterogeneous populations.

Isolate populations have been used to identify a number of putative loci and a number of disease genes associated with common diseases and quantitative traits such as asthma (Ober et al., 1998, Ober et al., 2000, Laitinen et al., 2001), schizophrenia (Moises et al., 1995), diabetes and body mass index (Hanson et al., 1998), blood pressure (Perola et al., 2000), ischemic heart disease (Pastinen et al., 1998), familial hyperlipidemia (Pajukanta et al., 1999) and multiple sclerosis (Kuokkanen et al., 1997, Kuokkanen et al., 1996). Of notable ophthalmic importance is the identification of LOXL 1 (lysyl oxidase-like 1) associated with exfoliative glaucoma in Iceland (Thorleifsson et al., 2007, Marx, 2007). Isolates have also contributed to our understanding of the genetics of age-related macular degeneration (Magnusson et al., 2006, Edwards et al., 2005), refractive error (Stambolian et al., 2004, Ibay et al., 2004, Stambolian et al., 2006, Wojciechowski et al., 2009, Biino et al., 2005) and glaucoma (Gencik et al., 1982, Plasilova et al., 1998, Morissette et al., 1995, Faucher et al., 2002, Baird et al., 2003, Baird et al., 2005a, Baird

et al., 2005b, Charlesworth et al., 2005, Craig et al., 2001).We planned to use the Scottish population isolate of Orkney to locate genes associated with quantitative traits related to primary open angle glaucoma.

3.3.2 THE SCOTTISH POPULATION ISOLATE OF ORKNEY

The Orkneys consist of an archipelago of islands off the north coast of Scotland. Separated from mainland Scotland by geography and for a few centuries, by history, Orkney shares many of the hallmarks of an isolate population such as Iceland or Finland. Section 3.3.2.1 provides a more detailed description of Orkney's geography and history, followed by a discussion of its genetic heritage. Today the islands of Orkney are connected to mainland Scotland, and to each other by a series of ferry and air links. However, even though travel to the archipelago and between the islands has improved considerably over time, sea and weather conditions are often inhospitable. Coupled with Orkney's distance from much of Scotland and the rest of Europe means that even today, Orkney remains somewhat isolated from her mainland brethren, and would have been considerably more inaccessible in the past. In addition to this geographical isolation, there is evidence that endogamy was common during the 19th and 20th centuries (Boyce et al., 1973) Emigration and reduced fertility has caused a steep decline in the archipelagos' population, with the population decreasing almost fourfold in size from a high in the 1860s to an all time low in 2001 (McQuillan et al., 2008, McQuillan, 2009). This reduced population size coupled with endogamy, means many marriages that have

occurred, have been between related individuals. The culmination of these factors means that Orkney will have reduced genetic diversity compared to more heterogeneous, urban populations.

Despite the high levels of emigration, it is not however uncommon to still find generations of the same family remaining in Orkney (McQuillan, 2009). Large pedigrees means that linkage methods as well as association strategies can be used during genetic analysis. This is further facilitated by the detailed demographic and genealogical information available about Orkney's inhabitants which allows pedigrees of current volunteers to be traced back for several generations (Roberts and Roberts, 1983).

When this study was being designed, a cross-sectional family based genetic study, the Orcades Study, had already been established in Orkney by Dr. James Flett Wilson and his team at the MRC Human Genetics Unit and the University of Edinburgh. The aim of the Orcades Study at that time was to map disease genes associated with quantitative anthropometric traits as well as QTs related to cardiovascular disease and some other biochemical parameters. Dr. Wilson's long-term plan was to extend phenotyping to include other quantitative traits such as bone mineral density. Rather than establishing a new study in a new isolate, our plan was to utilize infrastructure and volunteers already available from the existing Orcades Study and eventually, genome-wide data as well, to investigate quantitative traits associated with primary open angle glaucoma.

3.3.2.1 A BRIEF HISTORY OF ORKNEY

About 10 miles off the north coast of Caithness, lies the archipelago known as the Northern Isles of Orkney. Composed of around 70 small islands and skerries, the islands are divided into three main groups – the North Isles, the South Isles and Hrossey, which is also known as the Mainland (see figure 3.1). The number of islands that are populated has declined over the past century and only around quarter of these islands are now inhabited (Boyce et al., 1973). The capital of Orkney is Kirkwall, and along with Stromness, the only other major town in Orkney. Both are situated on Hrossey. The main islands that lie north of Kirkwall, known as the North Isles, are Shapinsay, Stronsay, Sanday, Eday, Egilsy, Westray, Papa Westray, Rousay, Wyre, Gairsay and North Ronaldsay. South of Kirkwall lie Flotta, Hoy, Graemsay, South Ronaldsay and Burray. The latter two islands are connected to the East Mainland by a series of barriers known as the Churchill Barriers, erected during the second World War to guard the natural harbor of Scapa Flow from enemy invasion.

The history of Orkney has been discussed extensively elsewhere (Davis, 2007, Omand, 2003, Schei, 2007, Thomson, 2008, Berry, 1986) and will be reviewed only briefly here. The history of the people of Orkney can be divided into three main periods. The first phase dates back to pre-history, from Mesolithic times until Norse invaders colonized Orkney when the second period begins. The final phase of Orcadian history commences when Scottish rule dawns in Orkney in the 1400s. These periods are discussed in more detail below.

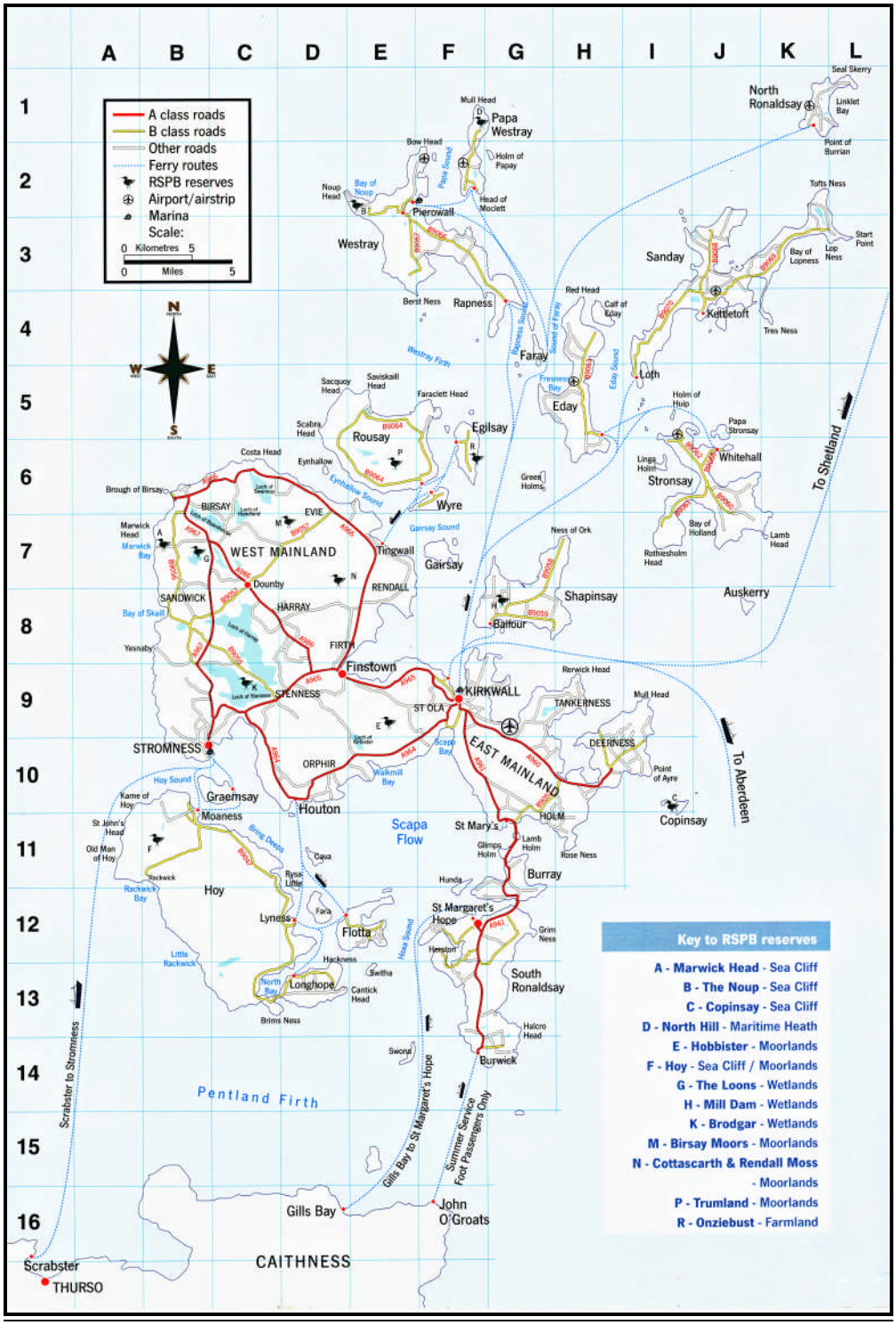


FIGURE 3.1: THE ARCHIPELAGO OF ORKNEY
(SOURCE: COURTESY OF *VISITORKNEY*, KIRKWALL, ORKNEY, KW15 1GU)

3.3.2.1.1 PRE-HISTORIC ORKNEY

Evidence for the occupation of Orkney extends to pre-history. (Davis, 2007, Omand, 2003, Thomson, 2008, Berry, 1986, Schei, 2007) However, archaeological evidence regarding the inhabitants of Orkney is scant in the time preceding the Neolithic period. The early inhabitants of Orkney were probably hunter-gathers whose existence is difficult to prove as they left little tangible evidence except for a few stone flakes and charred remains (Towrie, 2007). A burnt hazel nut discovered during excavations at a Bronze Age burial mound in Tankerness in 2007 was carbon dated to being between 6820 to 6660BC suggesting that Orkney may have been inhabited as far back as 7000BC. Despite the lack of remains preceding the Neolithic period, the assortment of structures from this era in Orkney is unparalleled in the United Kingdom.

The Neolithic Age in Orkney is believed to have commenced around 4000BC and continued for a period of around 2000 years (Schei, 2007). By this period, it is likely that the nomadic tribes of hunter gatherers had gradually developed into a more agricultural society requiring permanent dwellings. The red sandstone ubiquitous in Orkney would have been easy to fashion with stone age tools and proved to be a common building material at this time. One of the earliest settlements on record in Orkney as well as northern Europe is the Knap of Howar, which stands on the island of Papa Westray. This structure has been carbon dated to around 3600 to 3100BC. The sophisticated design of the house has suggested that there must have been earlier less developed farms which are yet to be discovered. Other famous Neolithic structures providing further insight into

pre-historic Orkney include Skara Brae (figure 3.2A), a cluster of stone houses unearthed around 1850 from their hiding place by storms moving the sand dunes that had cocooned them for several thousand years (Bailey, 2007, Schei, 2007); a number of burial tombs including Maeshowe on the Orkney Mainland and Midhowe on the island of Rousay (figure 3.2B); the henge monuments of Brodgar and Stenness (figures 3.2C and 3.2D) (Bailey, 2007, Schei, 2007).

FIGURE 3.2A: SKARA BRAE

FIGURE 3.2B: MAESHOWE

FIGURE 3.2C: STANDING STONES OF STENNESS

FIGURE 3.2D: RING OF BRODGAR

With the dawn of the Bronze Age, around 2000BC, there is evidence that there may have been a change in living and burial practices (Davis, 2007, Schei, 2007, Thomson, 2008). Though bronze artifacts have been discovered in Orkney, there is little proof of mining activity or the manufacture of such goods. Living and burial practices changed, away from communal living and burial such as seen in Skara Brae and Maeshowe, to more scattered communities and solitary cists. From around 1500BC, significant climatic changes are believed to have occurred. Temperatures declined and rainfall increased. The island may have become more isolated due to the increasingly inhospitable conditions. Great changes also occurred in the Iron Age of Orkney (circa 600BC – 715AD) that followed. Further impressive feats of engineering in the form of roundhouses and broch building occurred.

It was during this period that the first reference to Orkney is made in written work. The Greek geographer and explorer Pytheas from the area which is now modern Marseille, claimed to have circumnavigated the British Isles (c.325 BC) (Thomson, 2008). In his writing he allegedly made reference to an area of northern Britain he referred to as the "Orkas". It is also during this period, that the involvement of what is now considered to be a confederation of tribes, known as the Picts, with Orkney is thought to have occurred. The exact origin of the Picts remains unclear. The earliest recorded use of the name is in the year 296 in the works of Eumenius, pangyrist to Flavius Valerius Constantius (Ferguson, 1911). The world is believed to mean the "painted ones" from the Latin verb "to paint" (Davis, 2007). From 297 AD onwards, the Picts are referred to with increasing regularity in Roman records (Fisher, 1999). Some sources recognize two phases of Pictish culture (Davis, 2007). The first is an early pre-Celtic phase until around 100 AD witnessed by Romans such as the General, Agricola, around 82 or 83 AD. Agricola is known to have circumnavigated the British Isles, visiting Orkney at the time, though there are no records of Roman impressions of the archipelago. His son-in-law Tacitus recorded that Orkney was not only discovered but also conquered by the Romans (Thomson, 2008). Similar claims have been made in other classical writings. In 43AD, an Orcadian Chief allegedly surrendered to the emperor Claudius (Thomson, 2008). There is little other evidence to support these assertions, and it is possible these claims may have been greatly exaggerated to suggest that the Roman Empire stretched into the far reaches of Britannia.

83 AD witnessed the Roman slaughter of around 10,000 Picts as Rome attempted to secure more of Scotland. The battle site is believed to be somewhere within current Abderdeenshire and is recorded by the Romans as Mons Graupius. Despite this early victory, Rome never managed to crush the Picts and following a succession of smaller skirmishes, retired behind Hadrians' and Antoine's Walls to defend the northern frontier. It is possible that the losses incurred by the Picts during this time, both in life and in social stability, paved the way for the arrival and integration of the Celts (Davis, 2007). The second phase of Pictish culture in Orkney is characterized by Picts who embraced a Celtic language and way of life. The Picts of Orkney left behind a heritage of carved "symbol stones" , Celtic crosses, and some sources attribute broch building to the Picts (Davis, 2007) Another possible remnant of Gaelic influence is the continuing presence of place names such as "Pickaquoy", perhaps referring to the Picts of antiquity (Thomson, 2008). There are few written records during this time, but occasionally the archipelago is mentioned. During the visit of St. Columba of Iona to the Highlands, the presence of an Orcadian King in the court of Bridei mac Malelchon or Maelchu (King of the Picts c.555 to 585 AD) is mentioned (Ritchie, 2003, Schei, 2007). In various annals several expeditions against Orkney have been recorded – Aedan mac Gabrain (King of Argyll 574 to 608 AD), Bridei mac Bile (King of the Picts 872 to 693 AD) and in 709AD another Irish annal records a further war with the Orkneys. This period is thought to have forged closer links between the archipelago and the mainland and strengthened the Christian presence on the islands. By the advent of The Norse Period (c.780-1500 AD), Orkney was considered a Christian nation with an influential and wealthy church.

3.3.2.1.2 ORKNEY AND THE NORSE

The Viking Age dawned for most of Britain with the ravaging of Lindisfarne (793AD) followed by a succession of attacks on other British lands (Schei, 2007). The presence of the Norse can be traced by archaeological remains further in Orkney, to around 780AD. Whether the Norse established themselves in Orkney via mass genocide or by more peaceful means is still a matter of considerable debate. By around the 9th century, Orkney was an established Norse earldom, and the subsequent 300 years of Norse history (or legend) is covered in the *Orkneyinga Saga*, the saga of the Orkney Earls, a work believed to be written sometime around the 12th century by an unknown Icelander (Thomson, 2008). Following the battle of Largs in 1263, Norse influence in Scotland, including Orkney began to wane. In the 14th century Denmark, Norway and Sweden were united under a single monarchy in accordance with the Kalmar Treaty. The Earldom of Orkney officially remained under Nordic rule until 1468 when Princess Margrete of Denmark, the daughter of King Christian the I, married King James the III. As a part of the dowry, cash-strapped Christian, pledged Orkney and subsequently Shetland to James. By the 1470s, Orkney had been annexed to the Scottish Crown.

3.3.2.1.3 ORKNEY UNDER SCOTTISH RULE

By this period Viking dominance of the seas had been replaced by British mercantile and fishing fleets. Scots moved north, married with local Orcadians and gradually integrated into the religious, political and economic fabric of Orkney. Use of the Norn language spoken by the locals gradually declined. This was a period marred by rebellion (1528), poor leadership and two periods of famine (1631,1696) when thousands perished (Thomson, 2008). During this period, the bedraggled remnants of the Spanish Armada also floundered in the tempestuous seas off the North Coast. The survivors of the El Gran Grifon were shipwrecked on the Fair Isle. Other Spanish sailors found refuge and some subsequently married and settled in Westray. The descendents of these unions were named the "Dons" of Westray (Thomson, 2008, Anderson, 1988). On May the 1st 1707, the Act of Union united the Kingdoms of Scotland and England into the single Kingdom of Great Britain.

By the 1700s Orcadian farming was in much need of improvement. Orkney, for much of it's history, had initially been a farming community with subsequent forays into the fishing industry. The methods of farming being implemented were archaic, inefficient and impractical. Methods of "best practice" which were known at the time which would have improved yield were not implemented (Thomson, 2008). With the dawn of the 18th century other opportunities for employment arose. From the early 1700s, The Hudson Bay Company ships, bound to northern Canada, would sojourn in Stromness to hire much of the company's labor force. By the late 1770s, a large majority of employees in

the lower echelons of the company were Orcadian. Even the minimum monetary reward of joining the company was far greater than that of a farm servant in Orkney, so the appeal of a few years labor in Canada are understandable. Many Oracdians settled in Canada, sometimes with Native American wives. Some are said to have returned to Orkney accompanied by their wives and children, possibly introducing Cree connections into the Orcadian gene pool (Berry, 1986). Other vessels such as whaling fleets also recruited at Orcadian ports and prompted the emigration of young men from the islands. Further losses of men occurred during the Napoleonic Wars. Press gang activity was rife during this time and it is believed that around 2000 Orcadian men, about a 12th of the population at the time, served in the Navy. By the late 18th century, there was a boom in the kelp industry but by 1830, prices had dropped dramatically. This had serious economic consequences for much of Orkney and especially some islands of the archipelago such as North Ronaldsay which had gradually concentrated on the kelp industry at the detriment of farming. With the economic collapse of the kelp industry, interest was rekindled in farming. Once farming was revived with new knowledge and methods being integrated, prosperity blessed the islands once more and Orkney managed to retain more of her inhabitants.

The 20th century brought World War I and II and despite it's position in the far north of Britain, Orkney did not remain untainted by these wars (Schei, 2007, Thomson, 2008). The presence of a natural harbor, Scapa Flow, and its position in the North Sea, made Orkney a good of choice of navel base to protect northern interests in both World Wars. Life on the islands changed considerably during these times. With the influx of armed

forces and detention of prisoners of war on the islands, the population sometimes swelled to quadruple its norm. Various defensive structures were built, some which remain today. The landscape of Orkney was further changed when in the 1970s Orkney became involved in North Sea Oil industry, and built an oil terminal on the island of Flotta. Over the last few decades, the Flotta oil terminal has offered essential employment to the area and revenues from oil have helped bolster Orkney's economy. Though crude oil shipments from the North Sea to the terminal have declined over the last few years, the energy industry continues to contribute substantially to modern day Orkney.

3.3.2.1.4 ORKNEY TODAY

Orkney as it stands today, consists of a population of around 20,000 (Anon, 2006b, Anon, 2008). There has been a slight increase in the population since the last census in 2001 and the considerable decline in Orkney's population that had been predicted in the latter half of the 20th century has not occurred because of increased migration to the archipelago. Since the expansion of the European Community in 2004, there has been a substantial immigration of non-Orcadian individuals to the islands, with a relatively well balanced age profile. However, depopulation, especially of the smaller islands, remains a significant problem which public bodies are keen to address. There is a changing age profile in the archipelago, with a loss of young people between the ages of 16 and 24 to higher education and a relative increase in the population above the age of 55. Orkney

has one of the lowest average unemployment rates in Scotland (below 2%), with Orkney's economic activity rate in 2006 being around 4% above the national average (Anon, 2008). In 2003, 32.5% of the Orcadian work force were employed by the "health, education and public administration" sectors (Anon, 2006b). A further 25.3% were employed by "distribution, hotels and restaurants", construction employed around 9.6%, with "transport and communications" employing a further 8.4%. Though once farming and fishing were the mainstay of employment in Orkney, in 2003, the agriculture and fisheries sectors together only employed 7.2% of the total workforce.

Kirkwall is the capital of Orkney and can be found on the north coast of the Orkney mainland (see figures 3.1 and 3.3). The origins of Kirkwall can be traced back to Norse times, and from a single street in medieval times, Kirkwall has expanded to become the largest town and commercial hub of Orkney (Bailey, 2007). Kirkwall today is an eclectic mix of the old and new. The magnificent 12th century red sandstone edifice that is St.Magnus Cathedral, sharing its space with more contemporary architecture. Kirkwall now boasts a busy harbor and a thriving tourist industry as this once remote port is now easily accessible by both land and sea, with flights from all major Scottish Airports (Aberdeen, Edinburgh and Glasgow) as well as ferries from mainland Scotland and Shetland. The other Orcadian islands are linked to the Mainland of Orkney by ferry and flight services. It is in the burgh Kirkwall, the centre of Orkney's commerce and transport links, we chose as the base for the Orcades Eye Project along with other arms of the Orcades Study.

FIGURE 3.3: KIRKWALL, ORKNEY
(SOURCE: COURTESY OF *VISITORKNEY*, KIRKWALL, ORKNEY, KW15 1GU)

3.4 THE ORCADES STUDY

3.4.1 OVERVIEW OF THE ORCADES STUDY

The Orkney Complex Disease Study (Orcades) is an ongoing, family based, cross sectional study which was established by Dr. James Flett Wilson (JFW) and colleagues, of the University of Edinburgh and the MRC Human Genetics Unit to study genetic factors associated with cardiovascular diseases, a variety of anthropometric traits such as height and weight as well as a number of biochemical and hematological parameters in the Scottish population isolate of Orkney. Dr. Wilson's long term plan was to extend phenotyping to include a variety of other quantitative traits such as bone mineral density and intelligence quotient. The Orcades Study is now one of five population isolates of the European Special Populations Research Network (EUROSPAN), the aims and objectives of which are described on their homepage at the following URL: http://homepages.ed.ac.uk/s0565445/index.html. Other participants include CROAS, the Croatian Study, (Campbell et al., 2007, MICRO isolates in South Tyrol Study {Pattaro, 2007 #3090), the North Sweden Population Health Study (Johansson et al., 2009) and the Erasmus Rucphen Family Study (Pardo et al., 2005).

By 2005, the Orcades Study had recruited around 1000 volunteers for the measurement of the above quantitative traits. To enter the study, participants had to be over 18, with at least one grandparent from the north isles of Orkney. The Orcades Study at the time was housed in a van which acted as mobile unit, traversing the islands. A number of nursing

staff lead data collection and the collection of blood for genotyping, the analysis of various hematological and biochemical parameters, as well as storage for the future. By 2006, it was ready for a more permanent residence, and recruitment was to be extended to include individuals with a grandparent from the West Mainland as well as the North Isles.

3.4.2 FUNDING

Funding was required for the purchase of ophthalmic equipment to measure QTs, a salary for VKKK, travel, computer equipment and also static premises for the eye study as well as other arms of the Orcades Study. The existing Orcades Study was to provide clerical and administrative support, genetic data and nursing staff in the field to meet the University health and safety requirements. A research proposal was jointly written by VKKK and Alan Wright (AFW) with contributions regarding Orkney and Orcades Study provided by JFW. Funding was sought from a variety of sources (the Chief Scientist's Office of the Scottish Executive, Fight for Sight, the International Glaucoma Association, the Medical Research Council, The Scottish Hospitals Endowment Research Trust and the Ulverscroft Foundation). £30,000 in the form of a clinical fellowship was awarded to VKKK from the International Glaucoma Association (Ashford, Kent, TN24 8DH, **http://www.glaucoma-association.com/**) in September 2005. £250,000 was secured from the Chief Scientist's Office of the Scottish Executive

in the form of a project grant in June 2006. A final £20,000 was acquired by AFW from the MRC Human Genetics Unit towards capital equipment.

3.4.3 ETHICAL APPROVAL

Ethical approval had already been secured from the NHS Orkney Local Research Ethics Committee (LREC) by JFW in 2001 for the Orcades Study. This included approval for some ocular procedures (the measurement of refraction, intra-ocular pressure and ocular dimensions by ultrasound) but not some of the others we planned (visual acuity, optical coherence tomography, confocal scanning ophthalmoscopy and the examination of the eye using a slit lamp). We therefore sought an amendment to the NHS Orkney LREC approval to include these traits. Ethical approval was granted before the study commenced in 2007.

3.4.4 PREMISES

Once funding was secured, premises were sought on the main island of Orkney, the Mainland. At the time (July 2006), only one suitable property was available in Kirkwall. The property had previously been owned by the local Orkney newspaper, the Orcadian, and was situated on one of the main shopping streets of Orkney, Victoria Street (see map of Kirkwall, figure 3.3).

The location was ideal – in the centre of Orkney's capital Kirkwall on one of the main streets, adjacent a historic landmark (the St.Magnus Cathedral) and near the hub of Orkney's public transport links. Unfortunately the premises had been unused for over a decade and would require complete renovation (see figures 3.4A-C below).

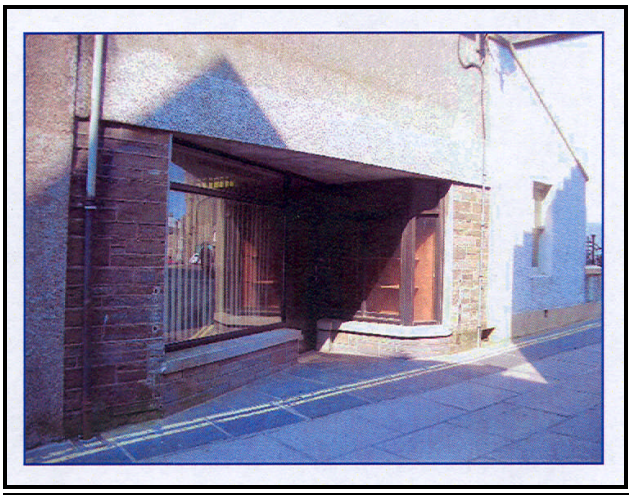


FIGURE 3.4A: EXTERIOR OF PREMISES*

FIGURE 3.4B: INTERIOR OF PREMISES (RECEPTION)

* Source: Photograph from the property schedule of Drever and Heddle, Estate Agents, Kirkwall, Orkney 2006.

FIGURE 3.4C: INTERIOR OF PREMISES (KITCHEN)

Negotiations with the owner regarding rent and renovations followed by the actual renovations occurred from the summer of 2006 until the spring of 2007. The plan was to convert the property into a custom made research centre which housed the eye project as well other arms of the Orcades Study. The negotiations were protracted and the renovations delayed for a variety reasons such as inclement weather so the start of the eye project, initially planned to be in September 2006, was delayed by several months. By April 2007, refurbishment was complete and it was finally possible for the equipment to be moved in and the eye project commenced. Figures 3.5A-C show the newly refurbished Orcades Study Centre and some of the common areas. Figures 3.5D-F shows the layout and the interior of the Orcades Eye Room.

FIGURE 3.5A: THE ORCADES STUDY CENTRE

FIGURE 3.5B: WAITING AREA

FIGURE 3.5C: STAFF COMMON ROOM/KITCHEN

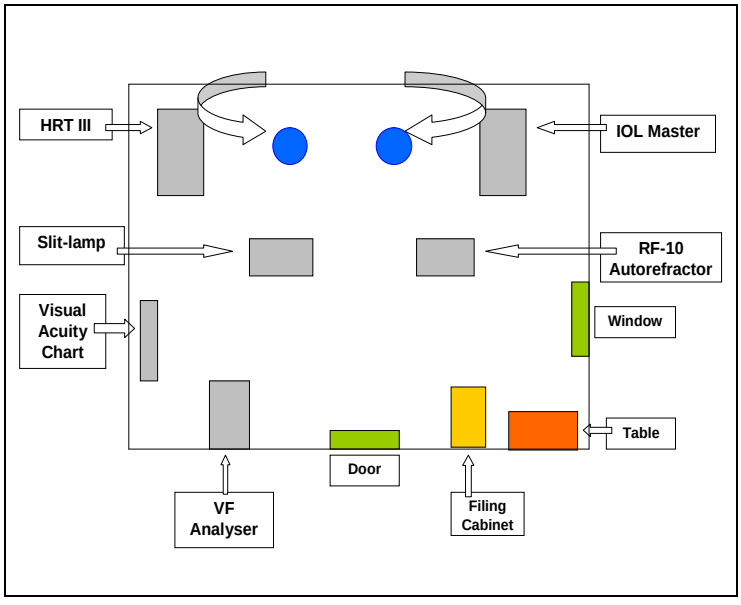


FIGURE 3.5D: LAYOUT OF EYE ROOM

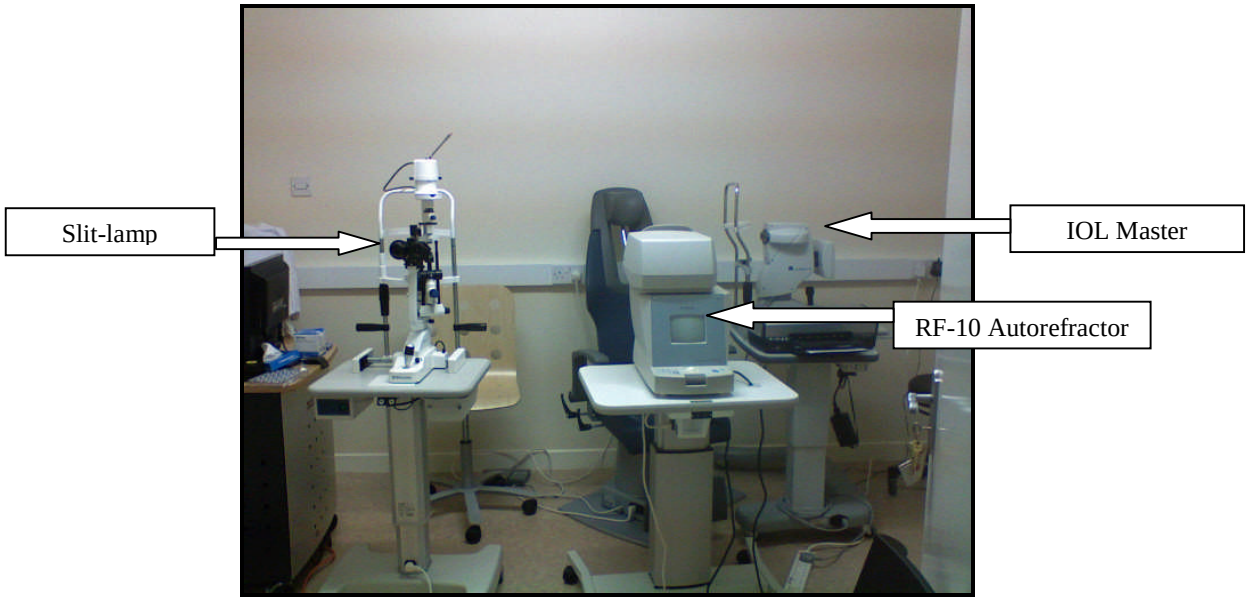


FIGURE 3.5E: ORCADES EYE ROOM

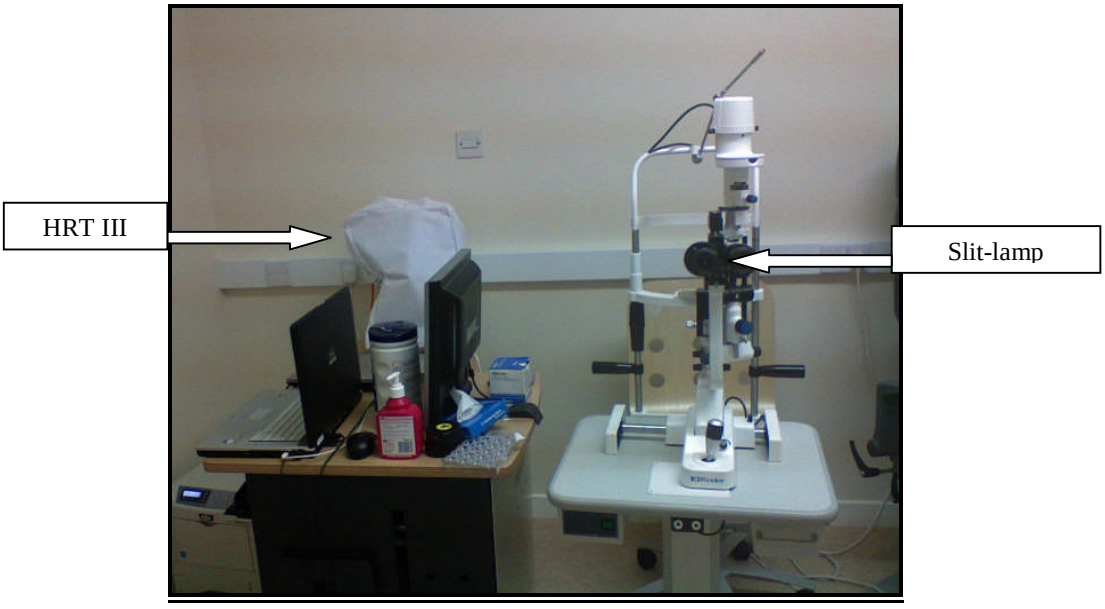


FIGURE 3.5F: ORCADES EYE ROOM

3.4.5 EQUIPMENT

Whilst the premises were being renovated, equipment was procured in accordance with the procurement protocols of the University of Edinburgh and the MRC Human Genetics Unit, Edinburgh with the aid of Mr. Andy Kordiak, Equipment Purchasing Manager of the College of Medicine and Veterinary Medicine of the University of Edinburgh. Specifically the following equipment was procured for the measurement of ocular biometric traits:

- Heidelberg Advanced IOPac Pachymeter (Heidelberg Engineering, Germany, supplier Haag Streit Ltd, Essex CM20 2TT) - for the measurement of central corneal thickness
- Keeler Slit lamp (Keeler Ophthalmic Instruments, Windsor SL4 4AA) - for the qualitative assessment of the eye and measurement of intraocular pressure
- Canon-RF 10 Autorefractor (supplier Haag-Streit Ltd, Essex CM20 2TT) – for the measurement of refraction
- Carl Zeiss IOLmaster (Carl Zeiss Ltd, Hertfordshire AL7 1JQ) - for the measurement of axial length
- Heidelberg Retinal Tomograph (Heidelberg Engineering, Germany Haag Streit Ltd, Essex CM20 2TT) - for the measurement of optic nerve parameters.

In brief, a report of equipment required and reasons for choice of equipment was drawn up and tenders sought from at least three vendors (Keeler Ophthalmic Instruments,

Windsor SL4 4AA, Carl Zeiss Ltd, Hertfordshire AL7 1JQ, Haag Streit Ltd, Essex CM20 2TT). The offers needed to meet delivery and installations dates (initially believed to be September 2006), maintenance and call out requirements and vendors had to be willing to accept the University of Edinburgh's terms and conditions for supply. Once offers had been obtained from the vendors, a final equipment selection was made and purchases arranged. All furniture and equipment except for the Carl Zeiss IOLmaster and the autorefractor were purchased via the University of Edinburgh. The IOLmaster and autorefractor were purchased through the MRC Human Genetics Unit with the aid of Mr. Eric Thomson. The documents and reports written for the procurement of equipment have not been included in the body of this thesis due to their bulk but are available for perusal.

3.4.6 SUBJECTS

By the time the Orcades Eye Project was being established, the overall Orcades Study had already recruited around 1000 participants for the Orcades Study as a whole. Entry criteria for the study at the time, was at least one grandparent from the North Isles of Orkney. This was subsequently extended to include individuals with one grandparent from the West Mainland. Many of these volunteers had already been evaluated for various anthropometric and cardiovascular traits and had blood taken for genotyping and the measurement of various biochemical parameters. The plan was for these volunteers to return for the measurement of ocular traits as well as other QTs such as bone mineral

density. The number of individuals required for a QTL study is difficult to estimate as statistical power depends on family structure of the sample which was yet to be described and also the size of genetic effects. Studies using 1000-2000 volunteers from isolates such as Finland and Iceland have effectively mapped diseases associated with QTLs such as schizophrenia and stroke. Hence we initially aimed to collect data on ocular biometric traits from at least 1000 volunteers from the population isolate of Orkney.

Clerical and administrative support was provided by the overall Orcades Study, and was headed by Ms. Kay Lindsay at the Department of Public Health Sciences of the University of Edinburgh. Invitations to the eye project were sent out centrally with a description of the project (see Participant Information Sheet for ORCADES Eye Study, appendix 1), a copy of the consent form (see appendix 2). The invitation provided information about the eye project and other arms of the Orcades study as appropriate. Clinics were subsequently set up once replies were returned. Initially individuals who had been the first to participate in the Orcades Study were sent invitations. The reason for this was two-fold. First, these early individuals were the ones most likely to have their genotyping completed during the study period. Second, these individuals were chosen to prevent "volunteer fatigue" as at least a year would have passed since they were last asked to participate in the Orcades Study. Each volunteer was assigned a unique identification number centrally, with the access codes known only to a very few individuals, so data collection and storage and analysis was anonymized. Along with other arms of the Orcades Study, the Eye project was advertised by word of mouth and

through the local media (see figure 3.6). So as well as using previously recruited volunteers, new participants were also actively sought for the project.

ORKNEY TODAY

Orcades Project sets its sight on learning from new eye study

by **BRIAN FLETT**

THE Orcades Project – which has been looking into the prevalence of life-threatening conditions such as heart disease, stroke, diabetes and osteoporosis within the Orkney population, and assessing to what extent they are hereditary – has branched out and is now also investigating eye diseases such as glaucoma.

While Dr Jim Wilson, from Holm, continues with the overall Orcades study, expanding the range of testing to include people whose grandparents come from the West Mainland, Dr Vidarshi Karunaratne, from Edinburgh University, is in charge of the eye study.

She explained the purpose of her research: "We would like to learn more about what causes glaucoma and other eye diseases, and in particular, to what degree genes influence the risk of disease. Glaucoma is the second most common cause of blind registration in Scotland. It affects 66 million people world-wide and it is the most common cause of irreversible blindness in the world.

"It is a very complicated disease. If you took all the blood vessels and nerves off – the eye would look like a golf ball. It needs a certain amount of pressure to maintain it. The optic nerve is basically the electrical conduit that connects the eye to the brain. What we think sometimes happens in glaucoma is that the pressure in the eye actually goes up and damages the optic nerve.

"What we've done is broken down glaucoma into its component parts and we're trying to find genes that might cause it. That is the main aim of the project."

The Orcades eye study clinic is also based at the former Orcadian offices at 9 Victoria Street in Kirkwall.

Dr Karunaratne points out that thanks to substantial financial backing from the Scottish Executive, the study team has got access to the highest of hi-tech testing equipment.

"We have a confocal scanning laser opthalmascope here – which is very sophisticated bit of equipment. We don't even have one in Edinburgh. Basically it scans the eye and makes a three-dimensional image of it. It is possible to get such amazing detail from it. We are using all this equipment to hopefully find genes which will lead to better interventions for glaucoma.

"At the moment, the NHS spends about £140 million a year on glaucoma and what is known as 'glaucoma suspects' because it is such a complicated disease that we find it very difficult to diagnose. So we hope to find better ways of diagnosing it as well and treating it, in the future."

The good thing about the Orcades eye clinic is that it only takes about as long as a routine eye test, and yet the detail gained from the scan provides all the supplementary information the project team needs for its research.

Dr Karunaratne explains: "These are all complex diseases. They are dependent on variants not in one gene, but in several genes, and we believe they all have some degree of environmental influence. Because we are using the same DNA scan, and the same pedigree information about large families as the volunteers on the original Orcades Study, it is a very efficient project to run.

"At the moment, we have funding for a year. So I will be up here for year and hopefully we will have a definitive number of about 1000 people taking part as a sample.

"What we ideally hope to get is to find genes to lead to diagnosis and treatment of glaucoma. An individual's data is entirely confidential, unless we find something which a GP ought to know about. Say their blood pressure is high, we might let their GP know, if the person agrees. That kind of thing. The data will eventually will be published in scientific journals, but everything is completely confidential."

She concluded: "People in Orkney have been absolutely wonderful so far. We are very much one team, under the banner of the Orcades Project. I work for Edinburgh University as well.

"Everyone who wants to volunteer receives a pack which contains information about osteoporosis – our bone study, our memory and thinking study, the eye study sheet and the original heart and vessels study. All you have to do is tick the part of the study you're interested in or all of them."

Anybody interested in volunteering for the Orcades eye study should ring 0131 651 1643 or e-mail: orkney@ed.ac.uk.

Information packs can also be picked up by dropping in past the new research centre at 9 Victoria Street, Kirkwall.

Orcades team seeks 1000 new volunteers

ORCADES STAFF AT THE FORMER ORCADIAN OFFICES: Dr Vidarshi Karunaratne, Dr Sarah Wild, Eileen Wombwell, Linda Smith, Fiona Wyatt, Dr Jim Wilson, Jessemay McDonald, Lorraine Anderson. Missing are Linda Hagan and Nicky Bichan.

■ continued from **Page 1**

The local volunteers who have taken part in the previous cardiovascular Orcades study don't have to reregister – their blood and DNA details are already on file. But in order to find 2000 people, the team needs to recruit twice as many volunteers as in the past study.

Dr Wilson indicated who will be eligible for the osteoporosis study.

"You either have to have one grandparent from the West Mainland or one grandparent from the North Isles. North Isles folk are still welcome to come forward. There are hardly 2000 folk altogether living in the North Isles, so we have to expand out. It was always my plan to move in this way. We will eventually recruit from the East Mainland too. We've been very delighted with the response we've had in the North Isles and we're hoping that we'll get a good response in the West Mainland, as well. Folk don't have to live in the West Mainland, as long as one of their grandparents is from the West Mainland. We are inviting the existing folk back and recruiting 1000 new people."

The bone thinning condition, osteoporosis, affects about three million people across Britain. Dr Wilson explained why he had chosen Orkney for the next stage of the Orcades project.

"I could have set up the osteoporosis study somewhere else, but because we already have people who are willing and we have their DNA, it is value for money to base it in Orkney. We're looking for families, really. We're hoping that people who come forward might suggest their brother, uncle, mother or their cousin, who quite possibly live locally as well. It is a genetic study and they depend on inheritance of things like bone density. Taking part obviously helps medical research. But they also get their own little health check, a bone scan, cholesterol check, their blood pressure checked and a diabetes check. So it is actually very useful."

Anybody interested in volunteering should ring 0131 651 1643 or e-mail: orkney@ed.ac.uk, or pick up an information pack by dropping in past the new research centre at 9 Victoria Street in Kirkwall.

FIGURE 3.6: ARTICLES ABOUT THE ORCADES PROJECT IN THE LOCAL PRESS, *ORKNEY TODAY* AND THE *ORCADIAN*

Volunteers who agreed to participate were assigned an appointment at the eye clinic. Initially these lasted around an hour and subsequently, this time was decreased to 45 minutes. At an eye appointment, each volunteer was greeted, the overall eye project and aims of the project as well as the examination process explained and informed consent obtained. Details of the examination protocol are discussed in subsequent sections.

3.4.7 GENOTYPING

All Orcades participants were genotyped using the Illumina Infinitum Hap300 platform (Illumina, San Diego). Data cleaning and quality control procedures were carried out by Dr. Veronique Vitart (MRC Human Genetic Unit, Edinburgh) and Dr. Ruth McQuillan (Department of Public Health Sciences, University of Edinburgh). Participants with call rates of less than 95%, SNPs with more than 10% missing, and SNPs failing a Hardy Weinberg threshold of p=0.0001 were eliminated. IBD sharing between all first and second degree relatives was assessed using the Genome program in PLINK (Purcell et al., 2007) and individuals falling outside expected ranges were removed from the study. Sex checking was performed using PLINK and individuals with discordant pedigree and genomic data were removed. On completion of data cleaning and quality control procedures, 186 individuals out of the 257 examined were available for genetic analysis.

CHAPTER 4

DATA COLLECTION, MANAGEMENT AND ANALYSIS

4.1 DATA COLLECTION

Data collection occurred at the Oracdes Eye Clinic at the Orcades Centre on Victoria Street, Kirkwall, Orkney (see figure 3.5A-F). Letters of invitations to join the eye project were sent by administrative staff at the University of Edinburgh to volunteers already participating in the Orcades study and to new volunteers who had expressed an interest in the project. Volunteers who agreed to participate were assigned an appointment at the eye clinic. Initially these lasted around an hour and subsequently, this time was decreased to 45 minutes. At an eye appointment, each volunteer was greeted, the overall eye project and aims of the project as well as the examination process explained and informed consent obtained. Details of the examination protocol are discussed in subsequent sections.

During the appointment the following data was collected:

- Medical history
- Drug history
- Social history

- Family ocular history
- Visual acuity
- Auto-refraction
- Axial length
- Keratometry
- White-to-white
- Central corneal thickness
- Intra-ocular pressure
- Optic nerve head parameters
- Qualitative slit lamp examination/ +/- eye movements depending on history.

All participants were asked a brief ocular and medical history (a detailed health and lifestyle questionnaire had already been taken for the majority of volunteers) covering current ocular/medical conditions, past medical and ocular history, drug and family history, and a brief social history to highlight tobacco and alcohol use. The reason for the collection of this information is two-fold. Certain medical conditons such as diabetes and hypertension, certain drugs such as tamoxifen, excessive tobacco and alcohol consumption are associated with characteristic ocular changes. As each patient was undergoing a complete qualitative slit lamp examination, if there are unusual findings during the study, they might be explained by past medical/drug or social history. For example, the presence of microaneurysms might be explained by an individual's history of diabetes. Furthermore, though this analysis will not be carried out in the initial stages of this study, the association between systemic disease and glaucoma as well as between

systemic disease and glaucoma related quantitative traits has been of interest for a number of years. For example, systemic diseases thant have been associated with primary open angle glaucoma including diabetes (Klein et al., 1994, Dielemans et al., 1996, Mitchell et al., 1997, Bonovas et al., 2004), blood pressure (Dielemans et al., 1995, Tielsch et al., 1995b, Bonomi et al., 2000b, Mitchell et al., 2004, Leskea et al., 2004, Leske et al., 2007a) and cardiovascular disease (Lee et al., 2006) and thyroid disease (Lee et al., 2004a) though these findings have not always been consistent across large cross-sectional population based studies as well as longitudinal trials (Kahn et al., 1977b, Leibowitz et al., 1980, Klein et al., 1993, Ponte et al., 1994, Leske et al., 1995, Tielsch et al., 1995b, Tielsch et al., 1995c, Wang et al., 1997, Ellis et al., 2000, Drance et al., 2001, Gordon et al., 2002, Geyer et al., 2003, Leske et al., 2003, Pache and Flammer, 2006, Leske et al., 2007a). Glaucoma related quantitative traits such as IOP have also been associated with systemic conditions. For example, hypertension has been associated with increased IOP (Dielemans et al. 1995, Nemsure et al., 2003b), and hypotension with decreased IOP (Klein et al., 2005) though again, these findings have not been consistent across studies (Leske et al., 1995, Somner, 1996). Hence gathering information regarding systemic disease as well as social history such as tobacco and alcohol use will allow the exploration of associations between glaucoma related quantitiative traits and systemic diseases such as hypertension and diabetes as well as gene-environment interactions in the future.

Patients with a history of previous ocular trauma, surgery or ocular surface disease, following examination by slit lamp, were excluded from the study if those factors were

likely to affect corneal integrity or ocular measurements. Ocular biometric traits were measured as follows.

4.1.1 MEASUREMENT OF VISUAL ACUITY (VA)

The most commonly measured form of visual function is visual acuity (American_Academy_of_Ophthalmology, 2002a, Elkington et al., 1999, Colenbrander, 2009). The first usage of the term is attributed to Francis Donders, a Danish professor of physiology who had subsequently developed an interest in ophthalmology (Colenbrander, 2009). In the mid 1800s, Donders defined visual acuity (VA) as a subjects' performance compared to a set standard – his concept of the "standard eye." At the time, the need for standardized vision charts had been recognized but the idea was yet to be realized. "Reading samples" to test vision were available, but they were beset with problems. Currently, the most commonly used chart for visual acuity measurement, the Snellen chart, was developed by Donders' colleague Herman Snellen, who Donders had recruited to develop a vision chart that integrated his concept of visual acuity.

The Snellen Visual Acuity Chart was revolutionary in it's time as Snellen used an external standard to define letter (or "optotype") size, so these charts could be reproduced by others. Snellen's method of assessing visual function rapidly gained popularity. Though it has undergone several incarnations, the Snellen Visual Acuity is still one of the most widely used methods of assessing visual acuity today. However, Snellen's Chart is not without it's issues – issues which make it less suitable for research

purposes (Hussain et al., 2006). Not all the optotypes on the chart are equally legible. The change in optotype size is random, the progression between lines is unequal, and the spacing between letters is not proportional which leads to increased visual crowding of letters in the lower lines. These problems were circumvented in the vision chart introduced by Bailey and Lovie in 1976, (Bailey and Lovie, 1976) – see figure 4.1 below.

FIGURE 4.1: KEELER 4M LOGMAR VISUAL ACUITY CHART

There are the same numbers of letters on each row, each letter is of equal legibility, spacing between letters and between rows is in proportion to letter size. Letter size shows geometric progression. The Bailey-Lovie chart with the sans-serif optotype designed by Louise Sloan, was popularized by the Early Treatment of Diabetic Retinopathy Study and has subsequently been used in many clinical studies (Ferris et al.,

1982, ETDRS, 1991, Beck et al., 2003, Colenbrander, 2009). This was also the method of visual function assessment we chose for this project.

VA was measured using a back lit Keeler “LogMAR 4m Test Type Chart” (Keeler, Windsor, United Kingdom). “LogMAR” refers to the method of recording visual acuity and is an abbreviation of the term logarithm of the “MAR” value. Louise Sloan introduced the concept of “visual angle notation” (Colenbrander, 2009). It refers to the ability to recognize each limb of an optotype which is 5 minutes of arc by 5 minutes of arc. The much used Snellen notation is essentially a ratio – where the numerator indicates the test distance and the denominator indicates at what test distance a “standard eye” would be able to view a letter. 1 minute of arc equals a Snellen visual acuity of 6/6 (1.0), 2 minutes, 6/12 (0.5) etc. The MAR value is the reciprocal of the Snellen visual acuity. What MAR stands for depends on the context of the assessment. It may represent the minimum angle of resolution if used in the context of optics, or the minimal angle of recognition in a clinical context or magnification requirement in visual rehabilitation. LogMAR is the logarithm of the MAR value and was introduced into clinical use by Bailey and Lovie. A visual acuity of 6/6 equals 0 in logmar notation, 6/12 is the equivalent of 0.3. The advantage of the logMAR as opposed to MAR notation, the scale is linear, the difference between each line of the chart being 0.1.

We used the recommended test distance of 4m in standardized illumination, following a protocol based on the suggestions made by the International Council of Ophthalmology, Visual Functions Committee and the Early Treatment Diabetic Retinopathy Study

(Visual_Functions_Committee, 1984). At a test distance of 4m, reading only the top line is scored at 1.0. Each subsequent line below is scored 0.1 less than the line above. Each letter on each line has equal legibility and is scored 0.02 (i.e.0.1/5). Each eye was tested in turn by covering alternate eyes with an occluder. If the top line could not be read, the volunteer was moved to a test distance of 1m and re-tested. A value of 0.6 was added to the score calculated as above to factor the new test distance. Volunteers able to only count fingers or detect hand movements at 50cm were assigned a LogMar equivalent of +2.00 and +3.00 respectively as suggested by Holladay (Holladay, 1997). Volunteers whose visual function was either perception of light or no perception of light would have their visual function scored as either "PL" or "NPL" respectively but no score assigned as both categories measure the perception of a stimulus rather than acuity.

Visual acuity is not a QT and the aim of this assessment is to quickly establish the global visual function of the eye. Some of the instruments described below require the patient to fixate on a target, and hence a minimum level of acuity is essential.

4.1.2 MEASUREMENT OF REFRACTIVE ERROR

The eye as an optical system has been studied since the time of Ancient Greece. One of the widely accepted theories is attributed to the Greek philosopher and physician Galen, who suggested that a psychic spirit that flowed through the optic nerve and globe, bathed the surroundings making matter visible (Katz and Kruger, 2009). By the 18th century,

due to the considerable advancement in optics that occurred during the Renaissance following the earlier work of the physicist Alhazen, the work of Johannes Kepler and Christopher Scheiner, it was established that the formation of the ocular image worked in a manner similar to the pinhole camera which had been described by Alhazen around the 10th century. It was understood even at that time that the image formed was determined by the refractive indices of the ocular media, the curvature of the various ocular surfaces and the distance between these elements, but a thorough investigation of these components was hampered by the lack of a techniques to measure them in vivo. We now know that the refractive power of the eye is roughly equivalent to the coordinated contributions of the refractive power of the cornea, the lens and the axial length of the globe (American_Academy_of_Ophthalmology, 2002b) and a multitude of methods are now available to measure these refractive components in vivo.

Autorefraction is an automated way of measuring the refractive state of the eye. The Canon RF-10 is both an automatic objective refractor (Canon 2007) and was used according to the manufacturer's instructions to measure the overall refractive power of the eye following VA measurements.

FIGURE 4.2: CANON RF-10 AUTOREFRACTOR

The optic principles of the Canon Autorefractor are shown in figure 4.3 (personal communication from Chris van Wijk, RF 10, Product Specialist, Eye Care Systems Department Canon Europa, Amstelveen, Netherlands). Rays of light are reflected by the retina of the volunteer's eye. The refractive state of the eye determines how these beams exit. In an emmetropic eye, beams that exit will be parallel to the visual axis. In myopic eyes rays will be convergent. Hyperopic eyes will show the converse – rays will be divergent. Some of the exiting rays are diverted and projected onto a TV camera where an image forms in the shape of a ring, where the sphere, cylinder and axis are calculated.

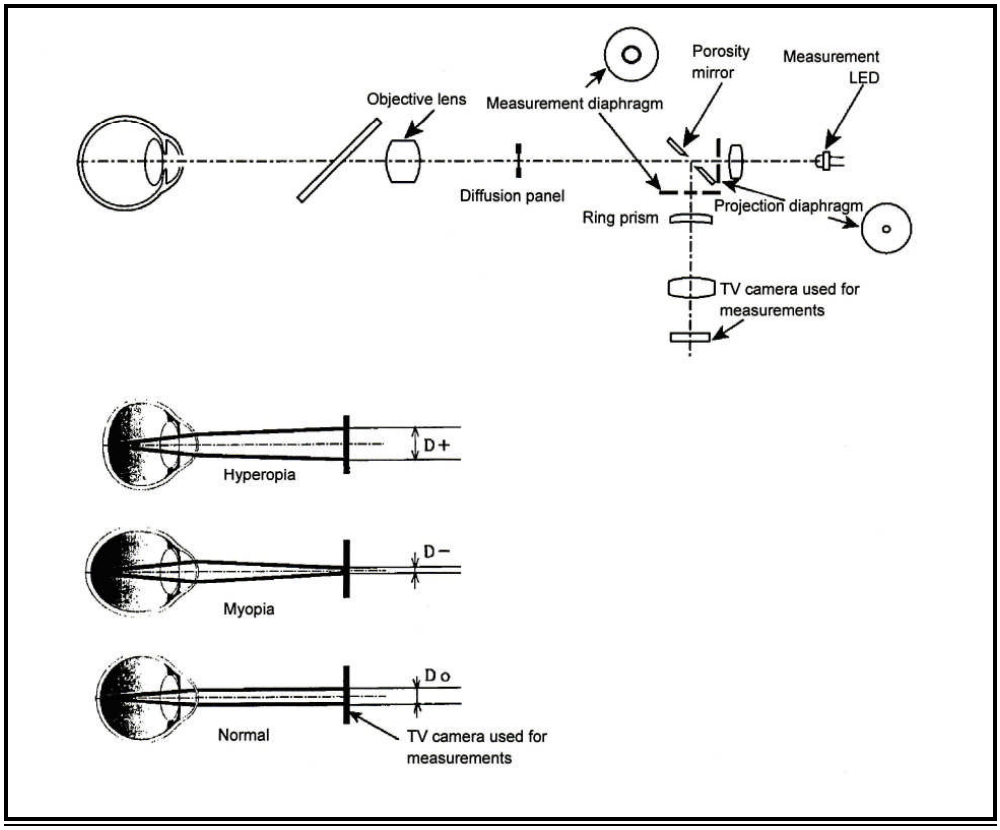


FIGURE 4.3: PRINCIPLE OF THE CANON RF-10 AUTOREFRACTOR
(SOURCE: C VAN WIJK, CANON AS ABOVE)

In brief, the volunteer was positioned as per instruction manual with their chin against the chin rest and the forehead on the forehead rest (see figure 4.4 below).

FIGURE 4.4: SUBJECT POSITIONING

The height of the chin rest was adjusted so that the volunteer's eye was in alignment with the adjustment mark. If this alignment was done correctly, the volunteer's eye would appear on screen. The volunteer was then asked to look at the fixation target (a red roof). If the pupil was off-centre, it was centered with the aid of the tracking ball and the "start" switch pressed. The instrument would then automatically perfect the alignment and completes the measurements. 3 sets of measurements were taken per eye and a standard value calculated from these measurements. A print out of the measurements was produced (see figure 4.5 below).

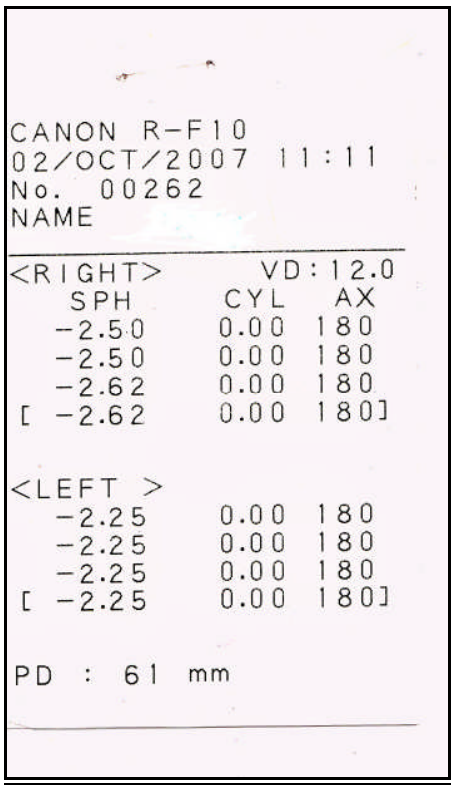


FIGURE 4.5: REFRACTION RESULTS FOR CANON RF-10

The first column of results (SPH) is a measure of the spherical value, the second column (CYL) shows a measure of the cylindrical value and the third column (AX) a measure of the axis. The standard value, shown in square parentheses, was entered into the data sheet. If the pupil was eccentric, the measurement was taken in manual mode. The procedure for eccentric pupils in brief is as above, but final alignment of the pupil with the alignment ring was done by the operator with the aid of the tracking ball.

4.1.3 MEASUREMENT OF AXIAL LENGTH, ANTERIOR CHAMBER DEPTH, CORNEAL CURVATURE AND WHITE-ON-WHITE

Techniques for measuring axial length (AL) have advanced considerably since the first methods involving X-rays and head clamps were described in the mid-20th century (Deller et al., 1947). AL is measured quite frequently in clinical practice as these measurements are required to calculate intra-ocular lens power prior to phacoemulsification. For many years techniques involving ultrasound were popular for making these measurements. However, over the last few years a new technique, using partial coherence inferometry introduced by Carl Zeiss (Carl Zeiss Meditech, Dublin) has been gaining popularity. Axial length measurements in this project were made using the technique employed by the Carl Zeiss IOLmaster (see figure 4.6 below).

FIGURE 4.6: CARL ZEISS IOLMASTER

The Carl Zeiss IOLmaster utilizes a technique known as partial coherence inferometry (also referred to optical coherence tomography/inferometry, Doppler inferometry), a reflectance technique analogous to conventional ultrasound. Rather than measure the velocity of ultrasonic waves, PCI measures the intensity of infrared light reflected back from ocular tissue interfaces. The velocity of light is too high so that echo delay techniques which measure ultrasound directly can not be employed and inferometry must be used instead.

Though PCI is a technique similar to ultrasound, the measurements made by either technique are not directly comparable. The dominant reflection in the acoustic axial length originates from the inner limiting membrane (ILM) of the retina, whereas in PCI, light is predominantly reflected from the retinal pigment epithelium. PCI has several advantages over ultrasound. The AL measured by ultrasound is approximates to the visual axis and extends from the anterior corneal vertex to the ILM. It is dependent on the operator positioning the probe correctly on the cornea, perpendicular to the corneal surface, in line with visual axis. Poor placement of the transducer, indentation of the cornea during the procedure will lead to axial length errors. Measurements made by optical coherence biometry are largely operator independent, non-contact with the measurement of axial length being dependent on the subject fixing on a target. PCI has high reproducibility (Carkeet et al., 2004, Hussin et al., 2006, Nemeth et al., 2003, Vogel et al., 2001) and shows little inter and intra-observer variability. Because of it's considerable advantages, the IOLmaster has now largely supplanted the A-scan except in the case of dense cataracts (Gale et al., 2006). The detailed use of the IOLmaster can

be found elsewhere (Zeiss, 2005). In brief, the procedure was explained to the subject and then the patient positioned appropriately, ensuring red alignment marks were level with the subjects' eyes.

FIGURE 4.7: SUBJECT POSITIONING FOR CARL ZEISS IOL MASTER

The visual axis of the subject is then checked by asking them to look straight at the yellow fixation light. At this point six illumination LEDs are visible on the monitor. These were centered around the pupil (see figure 4.8).

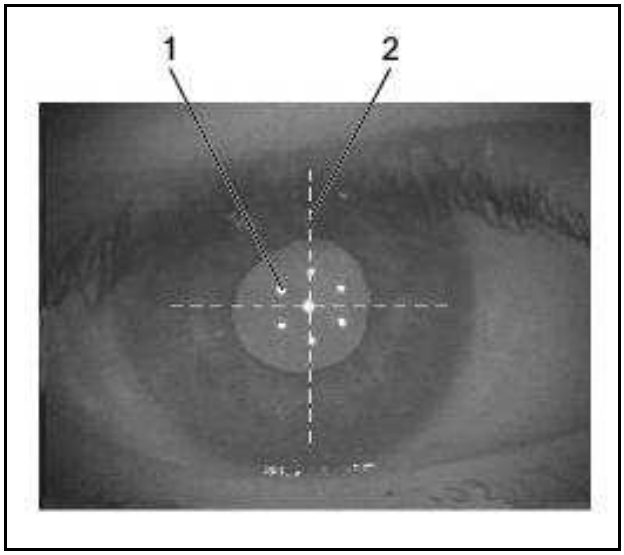


FIGURE 4.8: INITIAL POSITIONING OF EYE
(1= FOCUSING LIGHTS, 2=CROSS HAIRS)
(SOURCE: (Zeiss, 2005)

The machine was switched to axial length measurement mode using the joystick button. The subject was again asked to look straight ahead at the now red fixation light and open their eyes wide as possible. The alignment of the IOLmaster was perfected so that the reflection of the alignment light appeared as distinct as possible within the circle within the cross hairs.

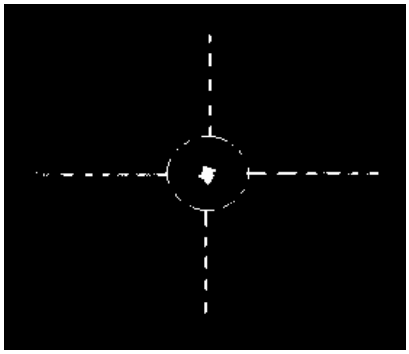


FIGURE 4.9: FINE ALIGNMENT OF EYE

The automated measurement system was started by pressing the joystick. An average of five measurements was taken per eye. The subject was then asked to lean back and rest and the measurements evaluated for quality. To be accepted the measurement had to have a valid signal curve (see figures below) a signal to noise ratio (SNR) of at least greater than 2.0 and chosen measurements had to be within ± 0.05mm of each other as recommended by the manufacturer. The figures below show examples of valid (figure 4.10) and poor (figure 4.11) signal curves.

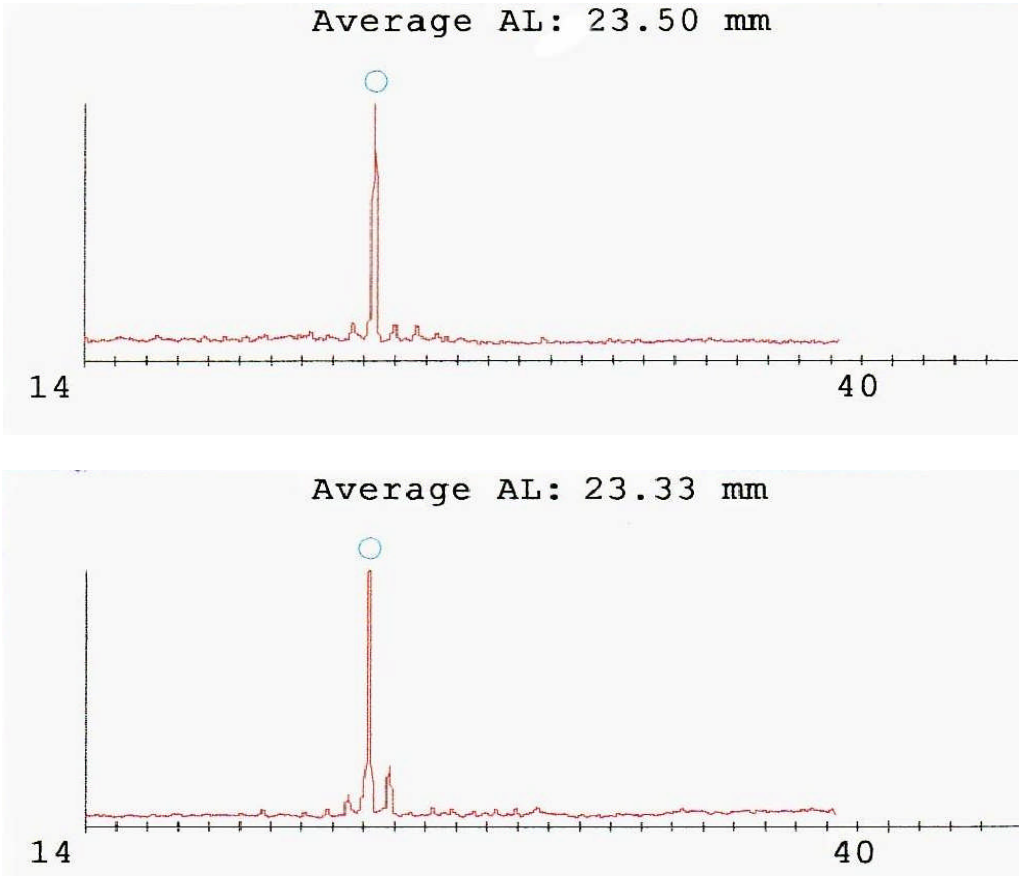


FIGURE 4.10: VALID SIGNAL CURVES

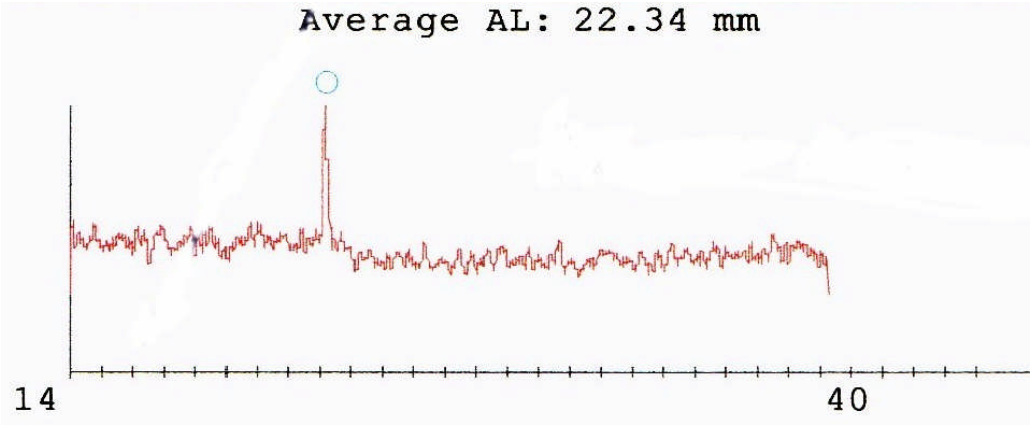


FIGURE 4.11: POOR SIGNAL CURVE

A minimum of 3 measurements per eye were required and the mean of these three measurements used as the final axial length.

Once axial length measurements had been evaluated and stored, the subject was repositioned and measurement of corneal curvature commenced. The eye was aligned as above, and the subject asked to fix on the now yellow fixation light and open their eyes as wide as possible. 6 peripheral measuring points appeared (see figure 4.12 below).

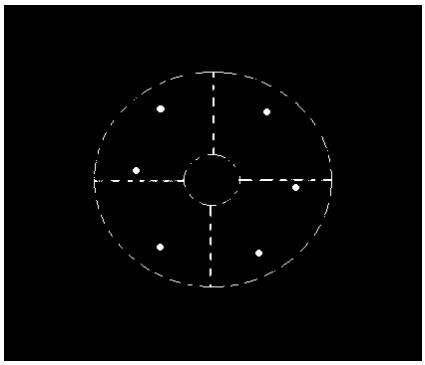


FIGURE 4.12: SETTINGS FOR KERATOMETRY

The IOLmaster was aligned so that the points were well focused and symmetrically placed within the circular cross hairs. The central area was not focused as it was included in the measurement. Pressing the joystick button started the measurement process. The software catches the image and then measures the distance between theses spots to calculate the radius of curvature (Berry, 2010). 3 measurements per eye were taken, and the mean of the three used as the final measurement.

Anterior chamber depth (ACD) was measured following corneal curvature. The subject was warned that a white light would appear from the side, but to concentrate on the yellow light in front of them. ACD in the IOLmaster was calculated as the distance from the anterior vertex of the cornea and the anterior vertex of the lens. The white point was focused so that it was distinct within the alignment rectangle on the screen, between the cornea and lens, adjacent to but not within the lens. Pressing the joystick button started the measurement process. ACD in the IOLmaster is measured using a slit illuminator principle (Berry, 2010). A beam of light in the form of a slit is projected onto the anterior segment at a known angle. By the position of the alignment rectangle, the instrument knows the placement of the cornea and lens. The corneal curvature would have been calculated previously and the depth of the anterior chamber is then computed using trigonometry. 5 measurements are taken, and the mean used as the final value.

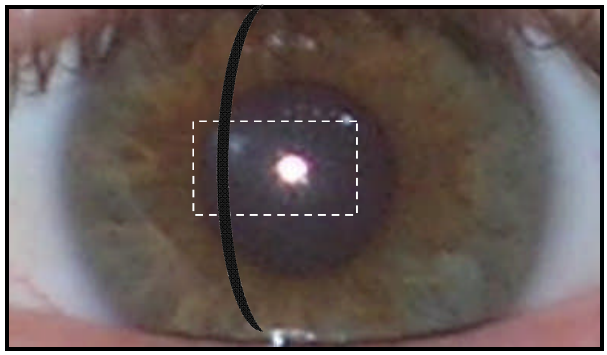


FIGURE 4.13: MEASUREMENT OF ANTERIOR CHAMBER DEPTH

After measuring ACD, "white-to-white" distance, was measured. Though sometime described as "corneal diameter" and interpreted as "anterior chamber diameter", white-to-white measurements represent the horizontal diameter of the iris. The subject was asked to look at the yellow light and open their eyes as wide as possible. The IOLmaster was adjusted so that the iris and pupil edge are in focus and the 6 focus points were centered on the cross hairs (figure 4.14). The measurement was commenced by pressing the joystick button. Once the measurement was taken, the markings appeared at where the device presumed was the edge of the iris. These were checked to ensure they were valid. The measurement was repeated three times and the mean value of the three measurements used.

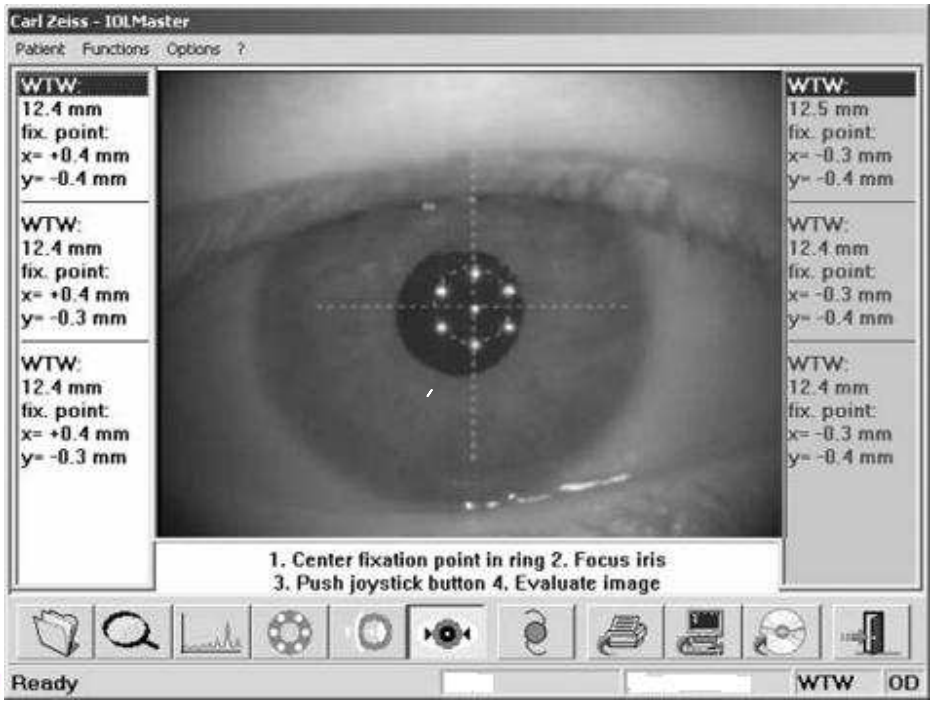
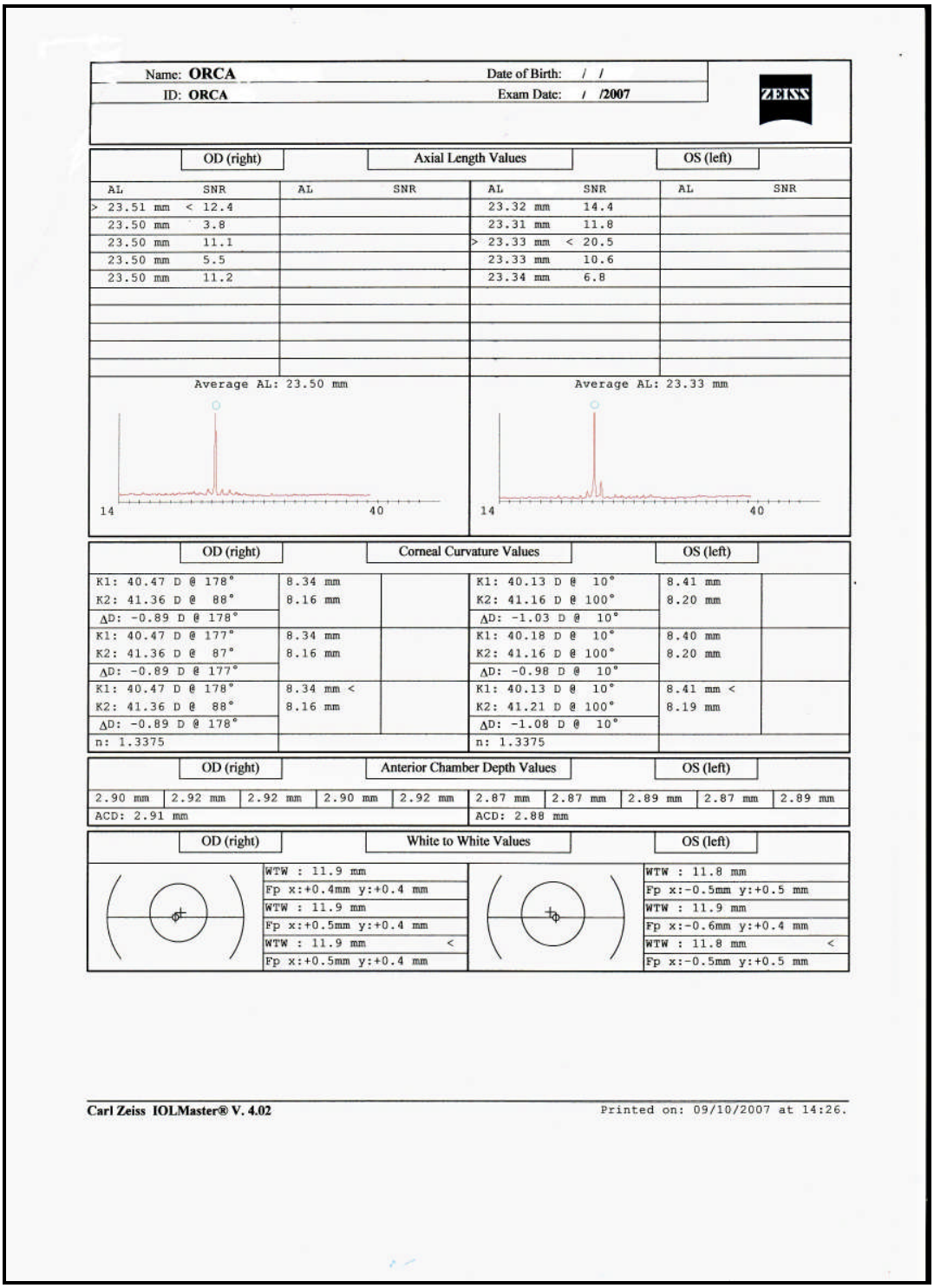


FIGURE 4.14: MEASUREMENT OF WHITE ON WHITE
(SOURCE: CARL ZEISS 2005)

The IOLmaster automatically determines which eye is being measured. Measurements of the other eye were taken by repeating the above procedure. A print out of the measurements was obtained (figure 4.15). Each results sheet contained the volunteer's unique identifier, date of birth and examination date (these have been removed from the figure to maintain confidentiality). Axial length values for both eyes are followed by keratometry parameters, anterior chamber depth measures and finally white on white.



Name: **ORCA** Date of Birth: / /
ID: **ORCA** Exam Date: / /2007

ZEISS

Axial Length Values

OD (right) AL	OD (right) SNR			OS (left) AL	OS (left) SNR		
> 23.51 mm	< 12.4			23.32 mm	14.4		
23.50 mm	3.8			23.31 mm	11.8		
23.50 mm	11.1			> 23.33 mm	< 20.5		
23.50 mm	5.5			23.33 mm	10.6		
23.50 mm	11.2			23.34 mm	6.8		

Average AL: 23.50 mm

Average AL: 23.33 mm

Corneal Curvature Values

OD (right)			OS (left)		
K1: 40.47 D @ 178°	8.34 mm		K1: 40.13 D @ 10°	8.41 mm	
K2: 41.36 D @ 88°	8.16 mm		K2: 41.16 D @ 100°	8.20 mm	
ΔD: -0.89 D @ 178°			ΔD: -1.03 D @ 10°		
K1: 40.47 D @ 177°	8.34 mm		K1: 40.18 D @ 10°	8.40 mm	
K2: 41.36 D @ 87°	8.16 mm		K2: 41.16 D @ 100°	8.20 mm	
ΔD: -0.89 D @ 177°			ΔD: -0.98 D @ 10°		
K1: 40.47 D @ 178°	8.34 mm <		K1: 40.13 D @ 10°	8.41 mm <	
K2: 41.36 D @ 88°	8.16 mm		K2: 41.21 D @ 100°	8.19 mm	
ΔD: -0.89 D @ 178°			ΔD: -1.08 D @ 10°		
n: 1.3375			n: 1.3375		

Anterior Chamber Depth Values

OD (right)					OS (left)				
2.90 mm	2.92 mm	2.92 mm	2.90 mm	2.92 mm	2.87 mm	2.87 mm	2.89 mm	2.87 mm	2.89 mm
ACD: 2.91 mm					ACD: 2.88 mm				

White to White Values

OD (right)		OS (left)	
	WTW : 11.9 mm		WTW : 11.8 mm
	Fp x:+0.4mm y:+0.4 mm		Fp x:-0.5mm y:+0.5 mm
	WTW : 11.9 mm		WTW : 11.9 mm
	Fp x:+0.5mm y:+0.4 mm		Fp x:-0.6mm y:+0.4 mm
	WTW : 11.9 mm <		WTW : 11.8 mm <
	Fp x:+0.5mm y:+0.4 mm		Fp x:-0.5mm y:+0.5 mm

Carl Zeiss IOLMaster® V. 4.02 Printed on: 09/10/2007 at 14:26.

FIGURE 4.15: RESULTS FROM THE CARL ZEISS IOLMASTER

Autorefraction, keratometry and measurement of axial length, occurred before pachymetry or tonometry to ensure the corneal surface was undisturbed.

4.1.4 General Ocular Examination, Measurement of Intraocular Pressure

The slit lamp is a high powered compound binocular microscope – that is it is constructed using several lenses, arranged to produce an image that has greater clarity and magnification than could be achieved with a single lens – see figure 3.5e (AAO 2002). Other lenses and mirrors integrated into the instrument ensure the image produced is upright and provide further magnification. The slit lamp is a standard instrument in clinical practice and allows the different optically transparent layers of the eye to be examined in great detail.

The aim of the slit lamp examination at this stage is to (1). Detect any ocular disease that would preclude contact measurements (tonometry and pachymetry), such as severe ocular surface disease (2). Highlight any pathology which make the volunteer' results inadmissible or difficult to interpret. For example, axial length measurements would be influenced by encircling bands used for retinal detachment surgery (3). Reveal any pathology which may influence intra ocular pressure. For example pseudoexfoliation or pigment dispersion.

Following slit lamp examination, and the installation of proxymetacaine and fluorescein, IOP was measured using Goldman applanation tonometry, the principles of which were described before. Three measurements were taken and an average of the three used before mydriasis.

4.1.5 EVALUATION OF THE OPTIC NERVE HEAD

In glaucoma, over a third of ganglion cell fibres can be destroyed before changes can be detected in the visual field (Harwerth et al., 1999, Kerrigan-Baumrind et al., 2000, Quigley et al., 1989). The evaluation of cup disc ratio is one the cornerstones of glaucoma management but subjective assessment of the optic nerve head shows high inter and intra observer variation (Varma et al., 1992). More automated methods now exist to analyze the optic nerve head. At the time this project was established, three main instruments were commercially available for this purpose – a confocal scanning ophthalmoscope, the Heidelberg Retinal Tomograph HRT (Heidelberg Engineering, Dossenhiem, Germany), an optical coherence tomograph (Status OCT, Carl Zeiss Meditec, UK) and a scanning laser polarimeter (GDX VCC, Carl Zeiss Meditech). These have been compared and discussed extensively elsewhere (Kwartz et al., 2005, Zangwill and Bowd, 2006). In brief, both OCT and GDx VCC concentrate on evaluating the retinal nerve fibre layer, with measurements either made along a parapapillary calculation circle in the latter or along a circle concentric with the optic disc, though not

in the immediate parapapillary zone. Only the HRT directly evaluates the topography of the optic nerve head.

The HRT (Heidelberg_Engineering, 2006) uses a 670nm diode laser to generate a three dimensional topographic image of the optic nerve head using a confocal scanning system. In brief, rather than flooding the entire specimen with light as in wide-field microscopy, confocal imaging uses a point source of illumination, usually one or more beams of laser, to scan across the object or specimen. Hence, only light in a narrow area surrounding a set focal plane can be detected. A series of optical sections are acquired for different positions along the optical axis, and these are constructed into a three dimensional image (for a comprehensive introduction to laser confocal microscopy see (Paddock, 2000)). Three models of HRT are available, the HRT I, II and III. At the time the eye project was being set up, published research related to either HRT I or II and the HRT III had only been available for commercial use for 18 months. It is in principle similar to the earlier models but has been designed to be more user and patient friendly. The project used the HRT III system to obtain quantitative optic nerve head parameters such as rim area, cup area and cup volume. Measurements obtained from the HRT III have been shown to have high degrees of reproducibility (Mikelberg et al., 1993, Rohrschneider et al., 1994, Miglior et al., 2002) and are able to detect subtle quantitative changes in optic nerve head morphology as well (Chauhan et al., 2000). Optic nerve dimensions are not merely diagnostic or prognostic indicators in glaucoma management. These parameters are true QTs and show high inter (and intra) individual variation (Sing

et al., 2000). It is still unclear however, which factors ultimately help determine these dimensions.

In addition, the HRT III allows the acquisition of peripapillary atrophy. As discussed in the introduction, for the last three decades, there has been increasing amounts of evidence to suggest that a relationship exisits between PPA and glaucoma (Primrose, 1970, Wilensky and Kolker, 1976, Nevarez et al., 1988, Buus and Anderson, 1989, Jonas et al., 1992, Jonas and Xu, 1993, Jonas et al., 1989, Jonas and Naumann, 1989, Araie et al., 1994, Lee et al., 2002, Xu et al., 2007c). Both PPA area and prevalence is greater in individuals with glaucoma than without (Jonas and Naumann, 1989, Jonas et al., 1989, Jonas et al., 1992, Jonas et.al 2002). There is also an association between PPA, glaucomatous optic neuropathy and visual field defects (Jonas et al.,1989, Araie et al., 1994). Progression of glaucomatous optic neuropathy have been associated with larger areas of ß- zone PPA, as well as PPA-disc ratio (Jonas et al.,1989, Araie et al., 1994) . These findings have not been universal (Nevarez et al.,1988, Puska et al., 1993, Quigley et al, 1992). Biomechanial multifactorial models of glaucomatous optic neuropathy as expounded by Belllaza et al. and discussed in chapter 1, provide a possible explanation for this association. Data from finite element modeling has demonstrated that peripapillary scleral thickness is one of the prinicple components that determines the effect of IOP generated stress on optic nerve head tissue (Bellazza et al., 2000). Decreasing wall thickness from 1.5mm to 0.5mm increased peripapillary scleral stress from 8 to 27 fold, increasing the potential to cause tissue damage. A study by Healey et al demonstrated that ß-PPA to have a high heritability (0.70, with a 95% CI of

0.54-0.83) (Healey et al., 2007). Hence it's possible association with glaucoma as well as evidence to suggest a strong genetic component in it's etiology, prompted us to collect data on this quantitative trait as well as other optic nerve head parameters.

4.1.5.1 IMAGE ACQUISITION

The optic nerve head images were aquired and evaluated according to the manufacturer's instructions as can be found in detail elsewhere (Heidelberg_Engineering, 2006). In brief, the volunteer's unique identification number, date of birth, gender and population affinity (Caucasian) was entered into the HRT III system (figure 4.16). Their refraction was also entered as the magnification, scaling as well as the absolute measurement results of the images are influenced by the refractive state of the eye. Corrective astigmatic lenses were used if the volunteer's cylinder was greater or equal to 1.0D.

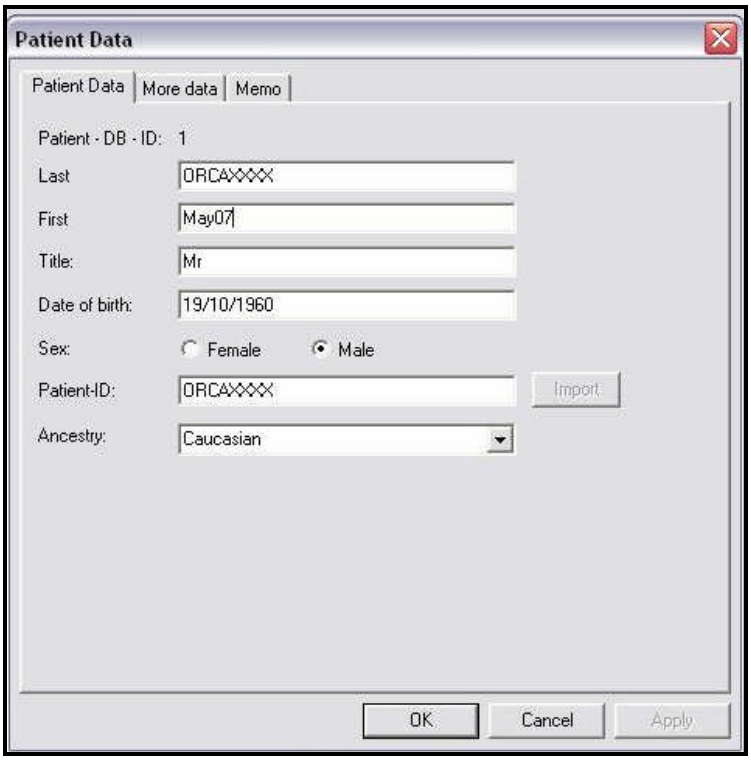


FIGURE 4.16: HRT III DATA ENTRY WINDOW

Once the volunteer's data was entered and confirmed with an "OK", the HRT III automatically switched into the image acquisition mode and a live window appeared. The volunteer was then positioned on the HRT III image acquisition module with their forehead against the headrest and asked to look at the HRT III fixation target. The laser beam of the acquisition module was adjusted so that beam was directly centered over the pupil with no light visible on the iris or in the case of small pupils, an even fringe of light framed the iris, and the camera. The camera was approximately 10 mm in front of the eye (just out-with the range of the eye lashes).

Once the beam was aligned, the volunteer was asked to look as their nose and then at the flashing green fixation light. After the volunteer's fixation was modified, the alignment of the laser was re-checked. These actions centre the optic nerve head in the image acquisition window. Finally, fine adjustments were made to achieve the best length of the image quality bar and the camera lens adjusted using a "bracketing" method – the objective was turned one position to the right or left. If the image increases in brightness, the procedure was repeated in the same direction after a few seconds. If the image decreases in brightness, the objective was moved in the other direction. This procedure was repeated until the best image is obtained.

Just prior to starting image acquisition, the above procedures and the positioning of the optic nerve head was rechecked, then the volunteer was asked to blink, keep their eyes wide open and the acquisition button pressed to start the process. If any warning message appeared after image acquisition, for example, due to eye movements, the process was repeated.

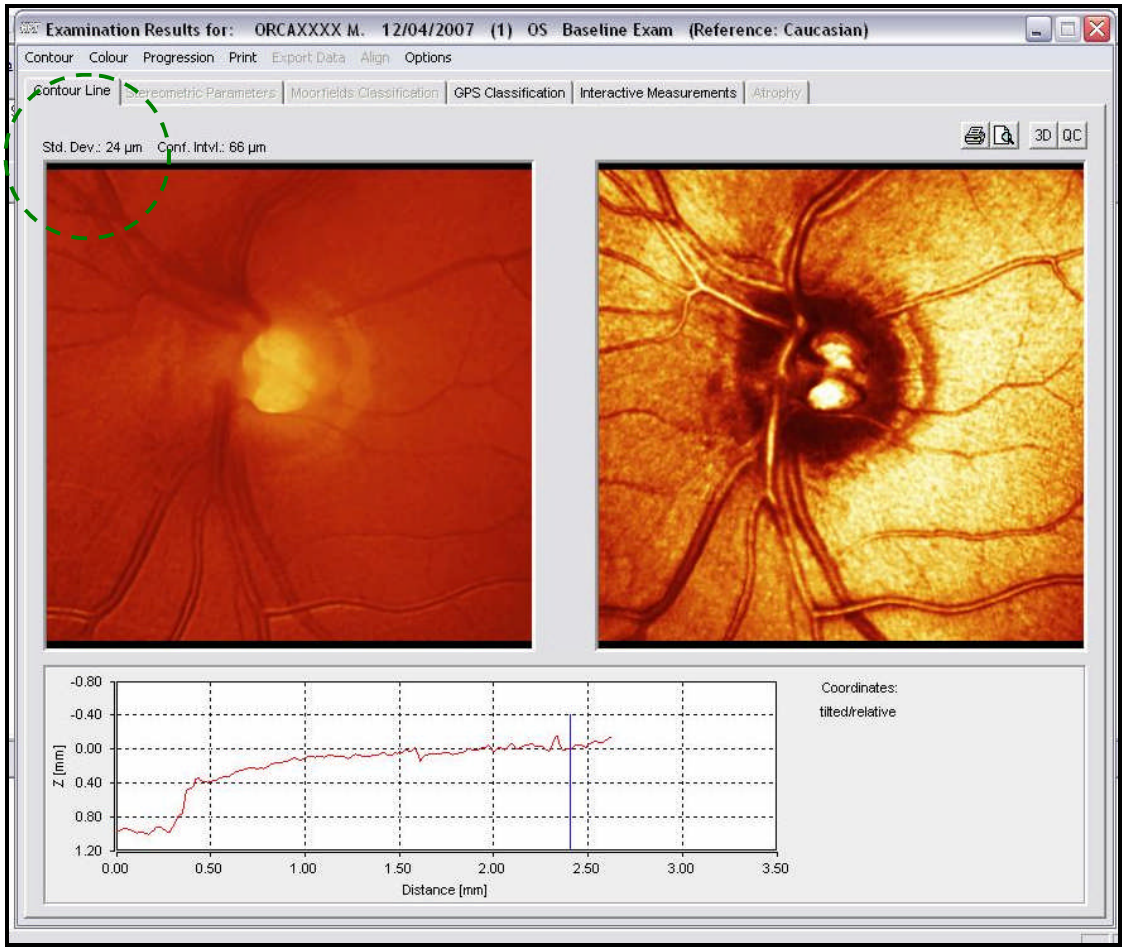


FIGURE 4.17: IMAGE OF ONH ACQUIRED BY HRTIII

Once the image was acquired (figure 4.17), the quality was checked. The quality of each image was assessed both subjectively – a visual analysis of the RNFL and ONH for shape, size, anatomical variation, the reflectance of a healthy RNFL, and also by assessment of the standard deviation of the topography image as described by the manufacturer (Heidelberg_Engineering, 2006). In brief, the standard deviation of the topography images can be found on the top left hand corner of the Analysis Centre window (see figure 4.17). This standard deviation represents a value calculated for each

pixel of the topography image, then averaged over the entire image. If there is little ocular movement during image acquisition, the images combined to form a mean image are similar. If this is the case, there will be very high image quality accompanied by a low standard deviation. If the standard deviation was greater than 30µm, the image acquisition was repeated. In some volunteers, due to the presence of other factors such as corneal scarring or lens opacities, it was not possible to improve image quality much further even after dilating the pupil. Images which scored a standard deviation above 50µm were not used in the final analysis.

4.1.5.2 ANALYSIS OF THE OPTIC NERVE HEAD STRUCTURE USING THE HEIDELBERG RETINAL TOMOGRAPH III

The Heidelberg Retinal Tomograph III (HRT III) allows the calculation of a series of stereometric parameters. For these parameters to be computed, a contour line around the ONH margin – the scleral or Elshnig's ring - must be drawn using the software provided as described (Heidelberg_Engineering, 2006). Briefly 5 contour points were placed approximately equidistance around the optic disc margin, and the contour defined (figures 4.18 and 4.19). The anatomic features of both reflectance and topography images in pseudo-color and in black and white such as the pallor of the scleral ring, the course of ONH blood vessels were utilized, as well as the surface height variation graphs as described in the operating instructions manual (Heidelberg_Engineering, 2006) to position the points and define the contour line.

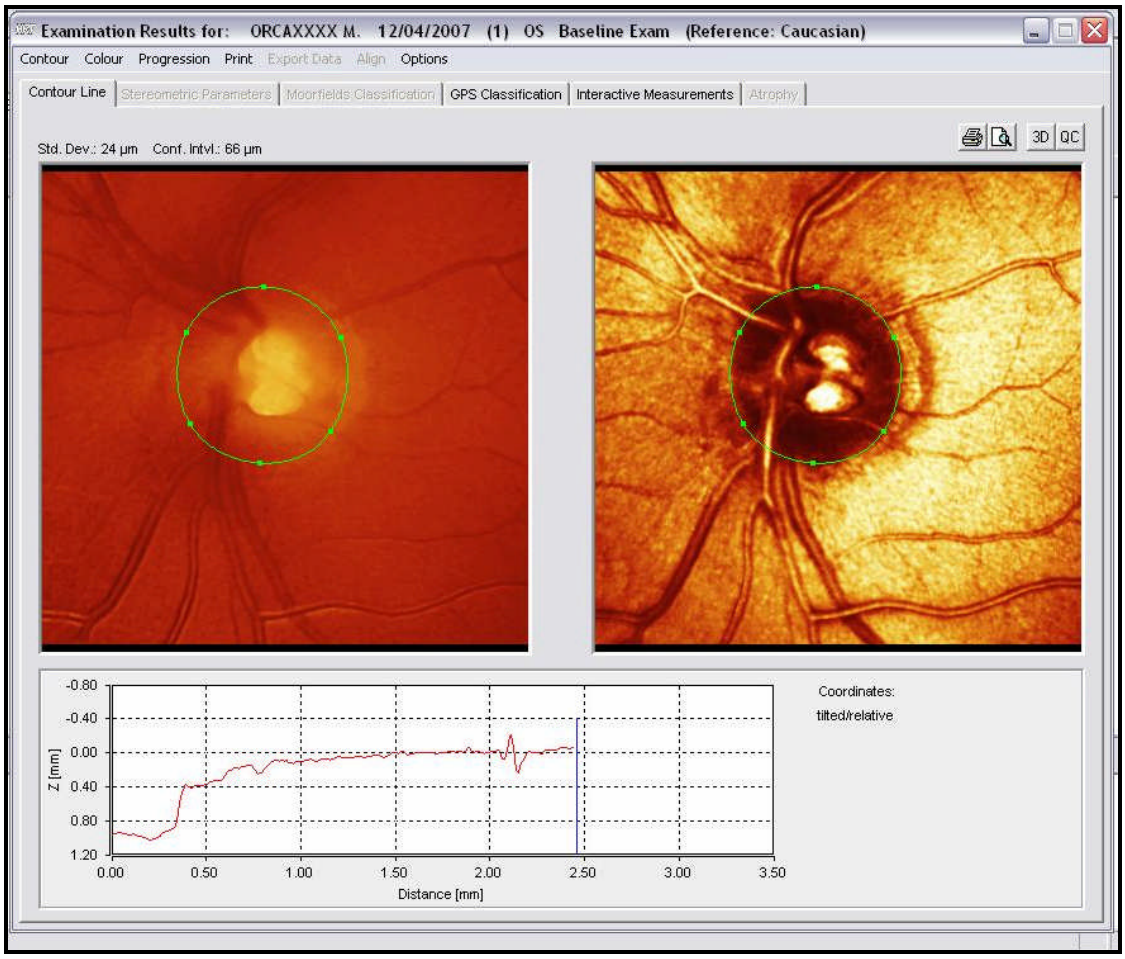


FIGURE 4.18: CONTOUR POINTS PLACED AROUND ONH MARGIN

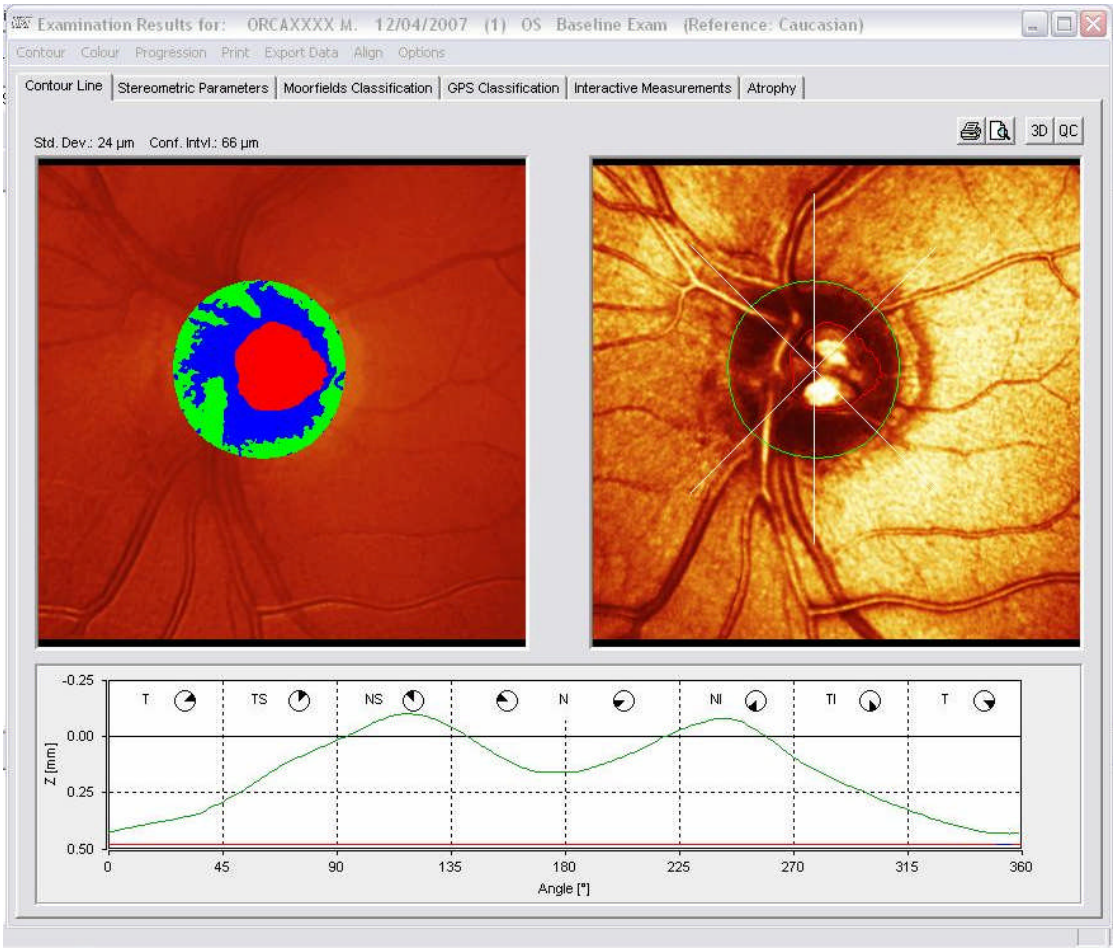


FIGURE 4.19: FINAL CONTOUR DEFINED

Care was made to ensure the contour line was drawn on the inside edge of the scleral ring, crescent and/or PPA but not far within the rim tissue. The contour line was positioned just outside the optic disc margin if there were any doubts as suggested in the instruction manual. If any section of the optic disc was obscured, for example, the nasal section by the presence of blood vessels, symmetry of the disc was assumed to define the contour line to a physiological oval or circular shape.

In order to calculate quantitative measures associated with PPA, a similar contour line enfolding peripapillary atrophy was drawn. Contour points were placed on the inner margin of PPA and drawn in a similar manner as described above. Images that were considered quite complex to define were discussed with an expert (Dr. Brian Fleck, Consultant Ophthalmologist, Princess Alexandra Eye Pavilion, Edinburgh) and a consensus for the contour or PPA line reached.

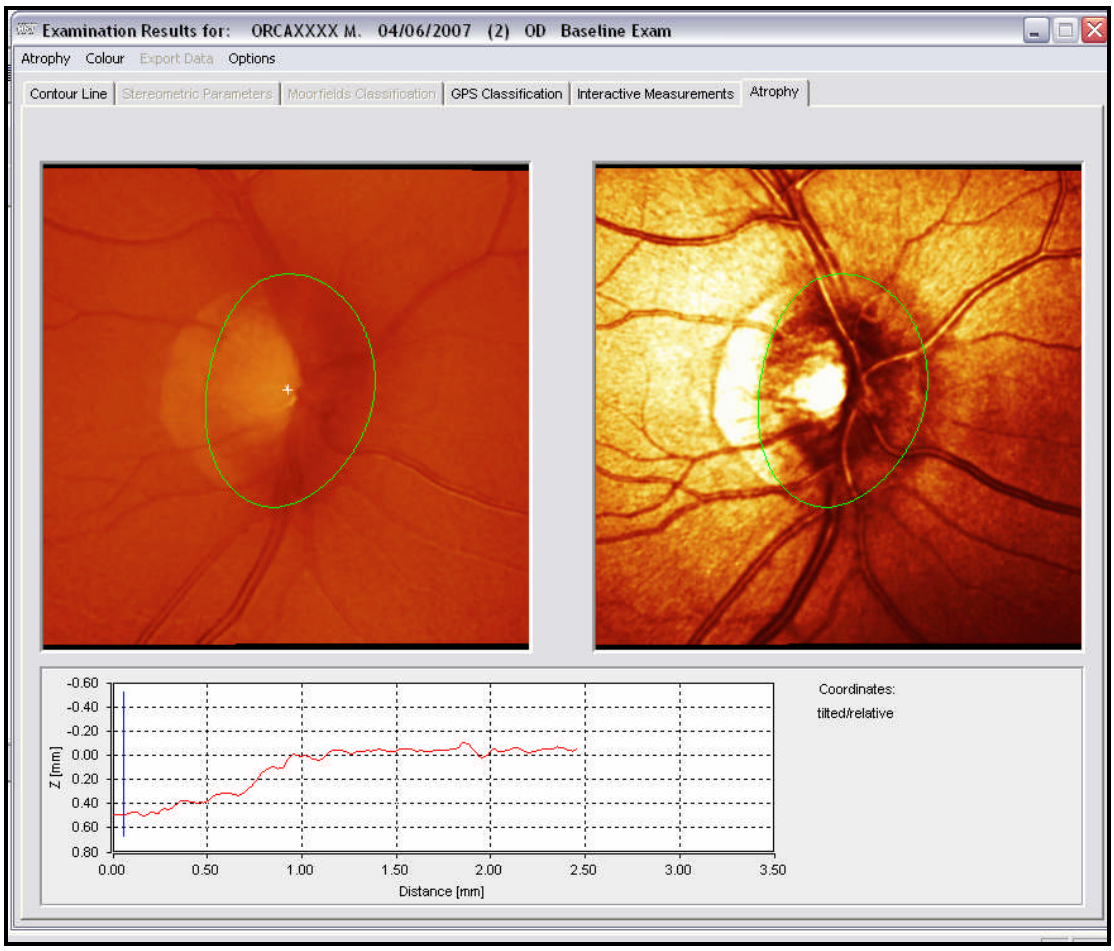


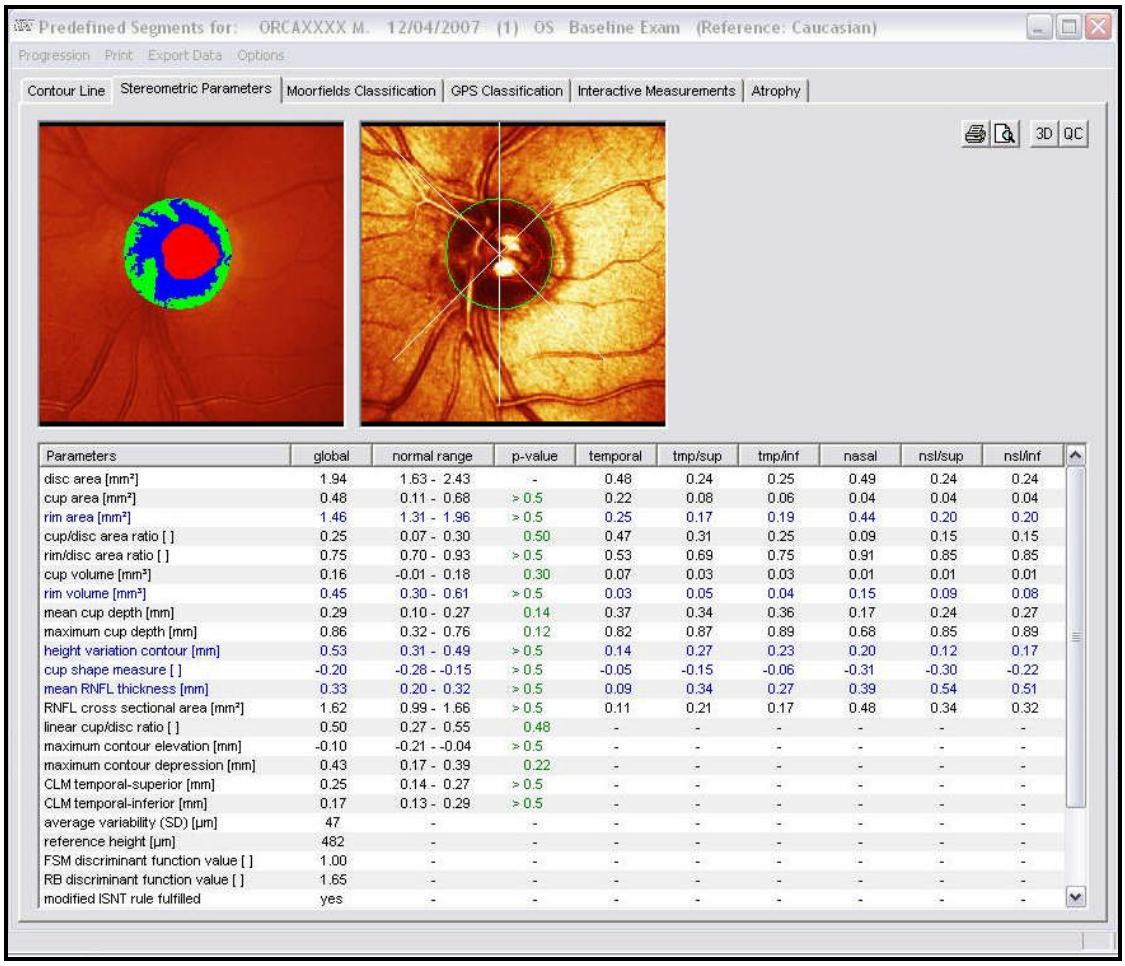
FIGURE 4.20: ATROPHY WINDOW

FIGURE 4.21: POINTS PLACED AROUND PPA

Once the contour line was drawn around the optic nerve head, the reflectance image was subdivided into six regions by the software - temporal (T), temporal superior (TS), nasal superior (NS), nasal (N), nasal inferior (NI), temporal inferior (TI). The convention for describing angles used by HRT III software is that 0° is defined temporally in both right and left eyes, then angular change progresses clockwise in the right eye and

counterclockwise in the left eye so that superior sectors are on average at 90°, nasal at 180° and inferior sectors at 270° regardless of the eye.

Once contour margins around the optic nerve head and PPA are drawn, the HRT III software defines a reference plane to delineate the separation between the optic cup and the neuron-retinal rim. The ideal theoretical position for the reference plane is beneath the retinal nerve fibre layer, corresponding to the papillomacular bundles as this remains relatively stable in glaucoma until the very end stages. The HRT III software attempts to approximate this position by placing the reference plane 50µm below the average height of the papillomacular bundle found between 350° and 356° of the ONH parallel to the peripapillary retinal surface. The area of the image ensconced by the contour line but above this reference place is considered to be neuro-retinal rim by the software. The area beneath this plane is considered to enclose optic cup. Using the reference plane the software calculates the retinal surface height which corresponds to RNFL thickness.



Parameters	global	normal range	p-value	temporal	tmp/sup	tmp/inf	nasal	nst/sup	nst/inf
disc area [mm²]	1.94	1.63 - 2.43	-	0.48	0.24	0.25	0.49	0.24	0.24
cup area [mm²]	0.48	0.11 - 0.68	> 0.5	0.22	0.08	0.06	0.04	0.04	0.04
rim area [mm²]	1.46	1.31 - 1.96	> 0.5	0.25	0.17	0.19	0.44	0.20	0.20
cup/disc area ratio [ ]	0.25	0.07 - 0.30	0.50	0.47	0.31	0.25	0.09	0.15	0.15
rim/disc area ratio [ ]	0.75	0.70 - 0.93	> 0.5	0.53	0.69	0.75	0.91	0.85	0.85
cup volume [mm³]	0.16	-0.01 - 0.18	0.30	0.07	0.03	0.03	0.01	0.01	0.01
rim volume [mm³]	0.45	0.30 - 0.61	> 0.5	0.03	0.05	0.04	0.15	0.09	0.08
mean cup depth [mm]	0.29	0.10 - 0.27	0.14	0.37	0.34	0.36	0.17	0.24	0.27
maximum cup depth [mm]	0.86	0.32 - 0.76	0.12	0.82	0.87	0.89	0.68	0.85	0.89
height variation contour [mm]	0.53	0.31 - 0.49	> 0.5	0.14	0.27	0.23	0.20	0.12	0.17
cup shape measure [ ]	-0.20	-0.28 - -0.15	> 0.5	-0.05	-0.15	-0.06	-0.31	-0.30	-0.22
mean RNFL thickness [mm]	0.33	0.20 - 0.32	> 0.5	0.09	0.34	0.27	0.39	0.54	0.51
RNFL cross sectional area [mm²]	1.62	0.99 - 1.66	> 0.5	0.11	0.21	0.17	0.48	0.34	0.32
linear cup/disc ratio [ ]	0.50	0.27 - 0.55	0.48	-	-	-	-	-	-
maximum contour elevation [mm]	-0.10	-0.21 - -0.04	> 0.5	-	-	-	-	-	-
maximum contour depression [mm]	0.43	0.17 - 0.39	0.22	-	-	-	-	-	-
CLM temporal-superior [mm]	0.25	0.14 - 0.27	> 0.5	-	-	-	-	-	-
CLM temporal-inferior [mm]	0.17	0.13 - 0.29	> 0.5	-	-	-	-	-	-
average variability (SD) [µm]	47	-	-	-	-	-	-	-	-
reference height [µm]	482	-	-	-	-	-	-	-	-
FSM discriminant function value [ ]	1.00	-	-	-	-	-	-	-	-
RB discriminant function value [ ]	1.65	-	-	-	-	-	-	-	-
modified ISNT rule fulfilled	yes	-	-	-	-	-	-	-	-

FIGURE 4.22: STEREOMETRIC PARAMETERS

Twenty two optic nerve head parameters are calculated by HRTIII software which is displayed in the stereometric parameters tab (figure 4.22). This includes areas for the optic disc, rim and cup, a mean and a maximum cup depth, and a value for cup volume. HRT III software also calculates a number of parameters for the PPA zone (see figure 4.29) including total area of PPA (in mm^2), total angular extent, total radial extent, and maximum distance from the contour line.

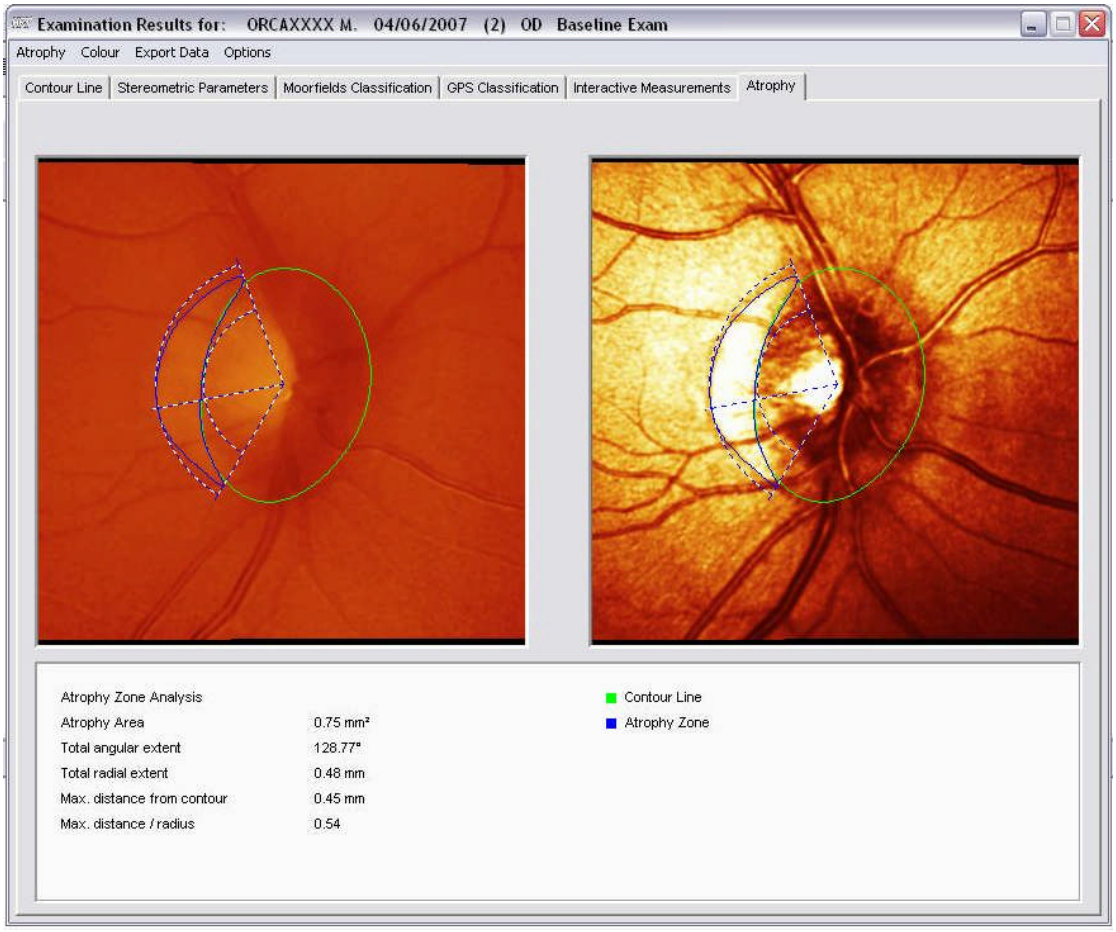


FIGURE 4.23: PPA PARAMETERS

Though not shown on the on-screen analysis, the HRT III also calculates the following parameters within 10 degree segments, with the first starting at 0 degrees temporal: 1. PPA area in mm². 2. Average width of the PPA zone (in µm) and the rim area (in mm²). For left eyes, the 10 degree sections are arranged in an anti-clockwise manner and for right eyes in a clockwise manner. The optic nerve and PPA parameters were exported into a XLS file compatible with Excel (Microsoft, Redmond, WA, USA).

4.1.6 MEASUREMENT OF CENTRAL CORNEAL THICKNESS

In ophthalmology pachymeters measure the thickness of the cornea. There are several different methods available to measure CCT. The instrument we used in the project, the Heidelberg Advanced IOPac hand held pachymeter, was based on an ultrasound technique (Heidelberg Engineering 2007). See figure 4.24 below.

FIGURE 4.24: HEIDELBERG ADVANCED IOPAC PACHYMETER

In brief, CCT was determined by measuring the time taken for echoes of ultrasonic waves to be reflected back from the anterior and posterior surfaces of the cornea. If the velocity of the waves through the cornea is known, then thickness can be calculated as velocity = distance/time (Airiani et al., 2006). Through CCT measurements made using the newer technique of partial coherence inferometry (PCI) show less inter and intra observer variability (Rainer et al., 2002, Nemeth et al., 2006), the high cost of PCI made

this method out with the reach of this project. The probe was positioned at the centre of the anaesthetized cornea, perpendicular to the corneal vertex, and acquisition commenced. The average of 5 consecutive readings was used.

4.1.7 VOLUNTEER FEEDBACK

At the end of the examination period, there was time for the volunteer to ask further questions regarding any aspect of the examination or project. If any abnormalities were found on examination, these were discussed with the volunteer, and permission obtained to write to their general practitioner (GP) if these finding were clinically significant. Referrals made regarding glaucoma suspects were accompanied by a summary of the measurements made in clinic and once a printer was installed, a copy of stereotactic parameters from the HRT III. This was to provide the local ophthalmology team with as much relevant information as possible to triage referrals efficiently. Subsequently, volunteers were also provided with a copy of their auto-refraction, axial length and intra-ocular pressure measurements (appendix 3). This was handed to them at the end of their period of examination, and the values on the sheet explained then and there. All letters to GPs were subsequently read and this protocol witnessed and approved by Dr. Brian Fleck in October 2007.

4.1.8 DATA COLLECTION INSTRUMENTS

All the data above except for the optic nerve parameters and IOLmaster measurements were entered into a data collection form. Information for the sake of future quality control of data such as the presence or absence of HRT/IOLmaster data and the reasons for such an absence was also collected. An initial data collection sheet was refined and a more efficient data collection sheet designed and used (see appendix 4) after a pilot study of 25 volunteers. Information from the IOL master was printed out and stapled to the back of the datasheet. The HRT III has a database which stores data generated by the image acquisition module. This was converted into an Excel (Microsoft, Redmond, WA, USA) format spreadsheet. Each data collection sheet was accompanied by a front sheet (see appendix 4), which contained the volunteer's details such as name, date of birth, contact details, GP details. This sheet was designed to be removed from the main data sheet at a later date to maintain anonymity of the volunteer.

The data collection form was designed to minimize free text and maximize the use of binomial variables (yes/no) to facilitate both data entry and future analysis. To facilitate accurate data entry, in general questions were placed on the left hand side of the page and responses to these questions on the right, divided by a long vertical line. Similar responses were placed along the same longitudinal. e.g. All "No's" to questions were placed beneath each other, so the operator's eye would follow the data quickly and was not forced to search a large page for information. A field for free text was included at the end of the sheet so that any extra information could be recorded.

4.2 DATA STORAGE

The data from the datasheet was entered into a database designed using Access 97 (Microsoft, Redmond, WA, USA).

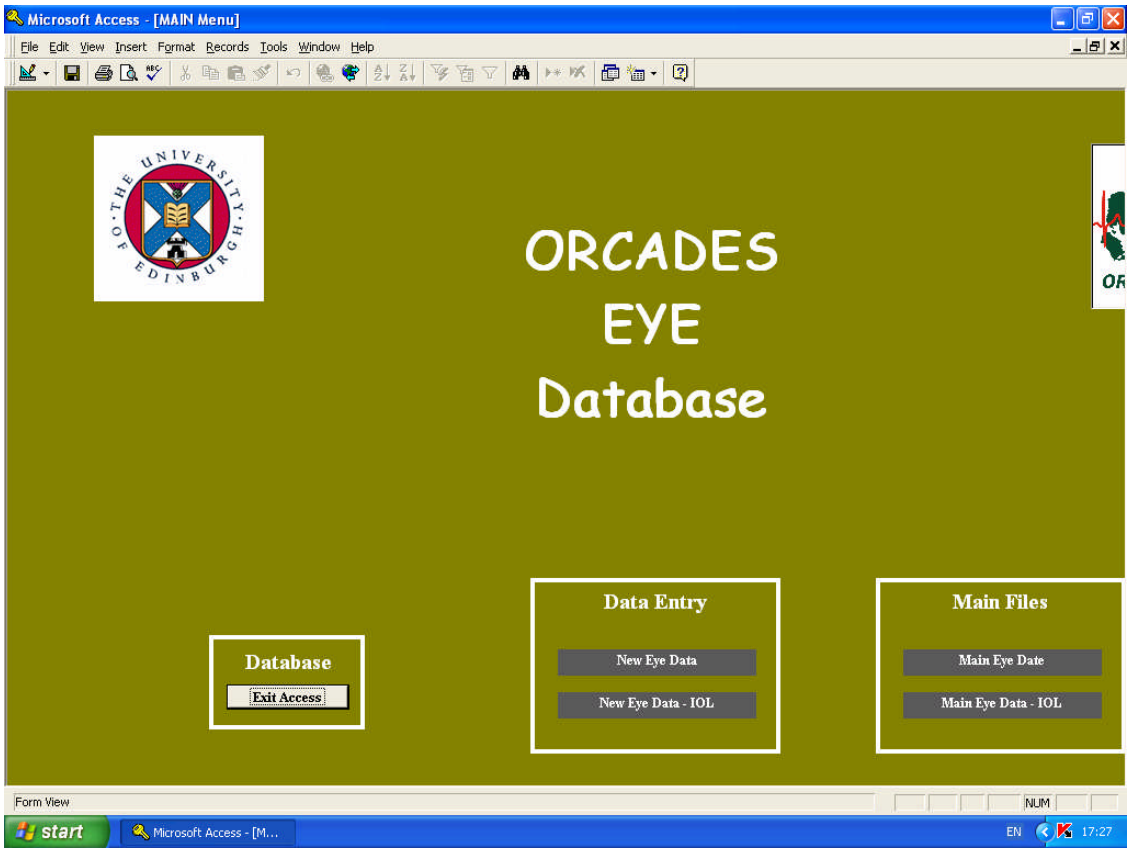


FIGURE 4.25: ORCADES EYE DATABASE

This dated version of Access needed to be used to ensure the Eye Database was compatible with other Orcades databases, all of which were based on Access 97. I

designed the initial database but Ms. Lesely McGoohan, (Department of Public Health, University of Edinburgh) kindly created and validated the first version. I subsequently modified the initial code and refined the database, and completed the final validation. This was done by entering 10% of the total datasheets, then double checking the entries by hand and double checking any calculations by hand. Due to the limited capacity of Access 97 databases, two databases had to be created – the first for the entry of all quantitative and qualitative data except for data from the IOLmaster and HRT III, the second for the entry of IOLmaster data. The HRT III had its' own database.

The entry forms of the access database were designed to mirror the data sheet (including the IOL master form), with questions and answers following the same format to assist accurate data entry.

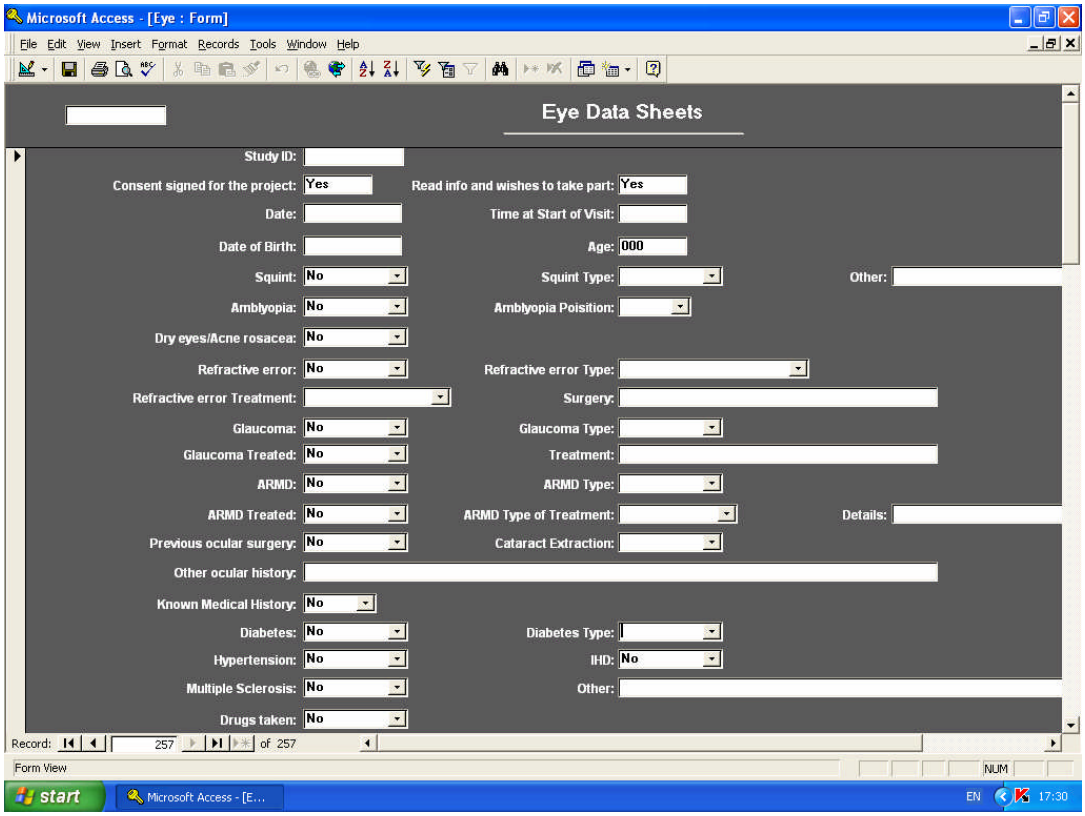
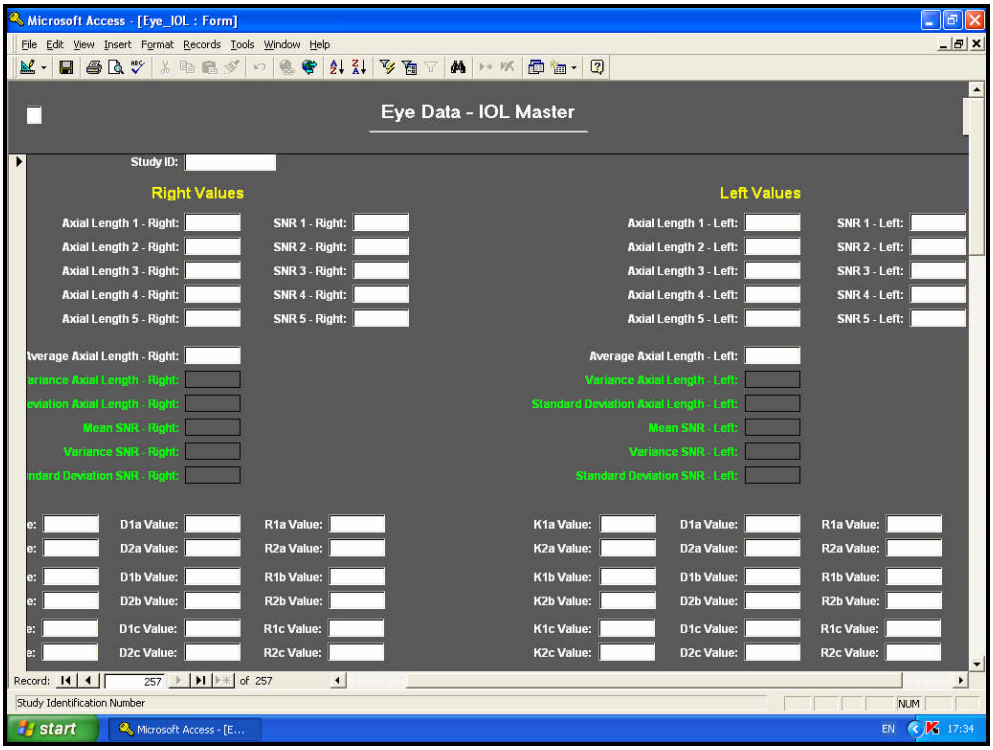


FIGURE 4.26: ORCADES EYE DATABASE – VISUALLY RESEMBLES DATA COLLECTION FORM



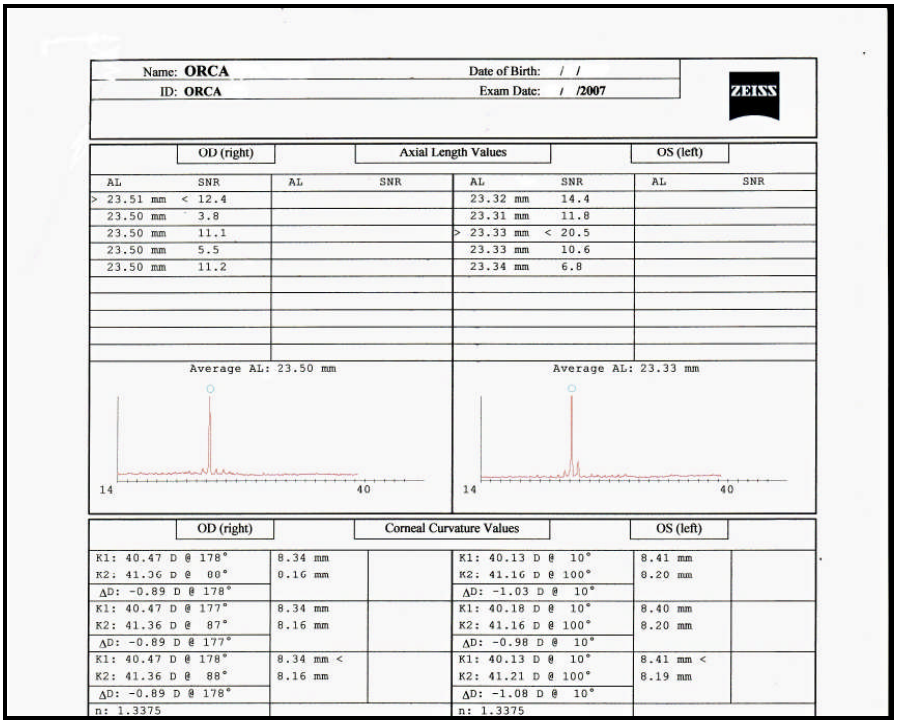


FIGURE 4.27: ORCADES EYE DATABASE – RESEMBLES IOLMASTER RESULTS SHEET

Volunteers were identified by their ORCA number. Each data variable was assigned a unique descriptive identifier. For example, the identifier for unaided visual acuity is VA_UN. The majority of fields consisted of either of drop down menus where answers merely need to be highlighted, or numerical fields. A minority of fields were designed for free text.

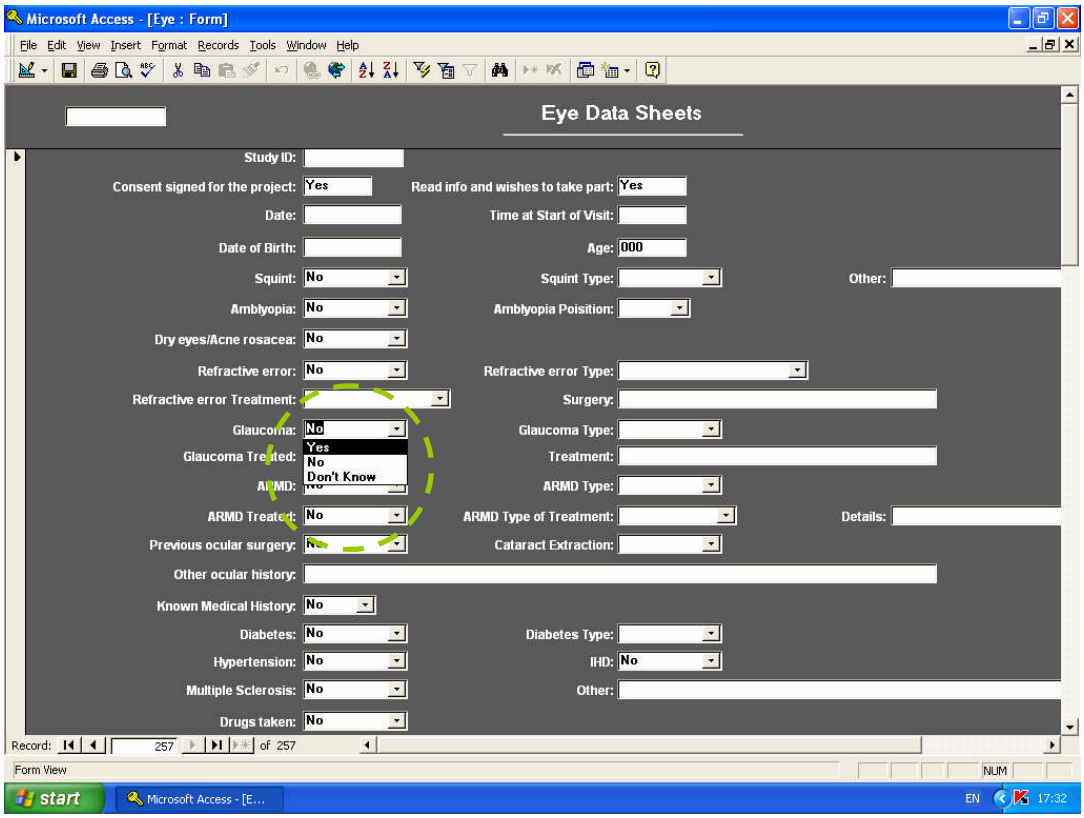


FIGURE 4.28: ORCADES EYE DATABASE – BINARY DROP-DOWN MENU

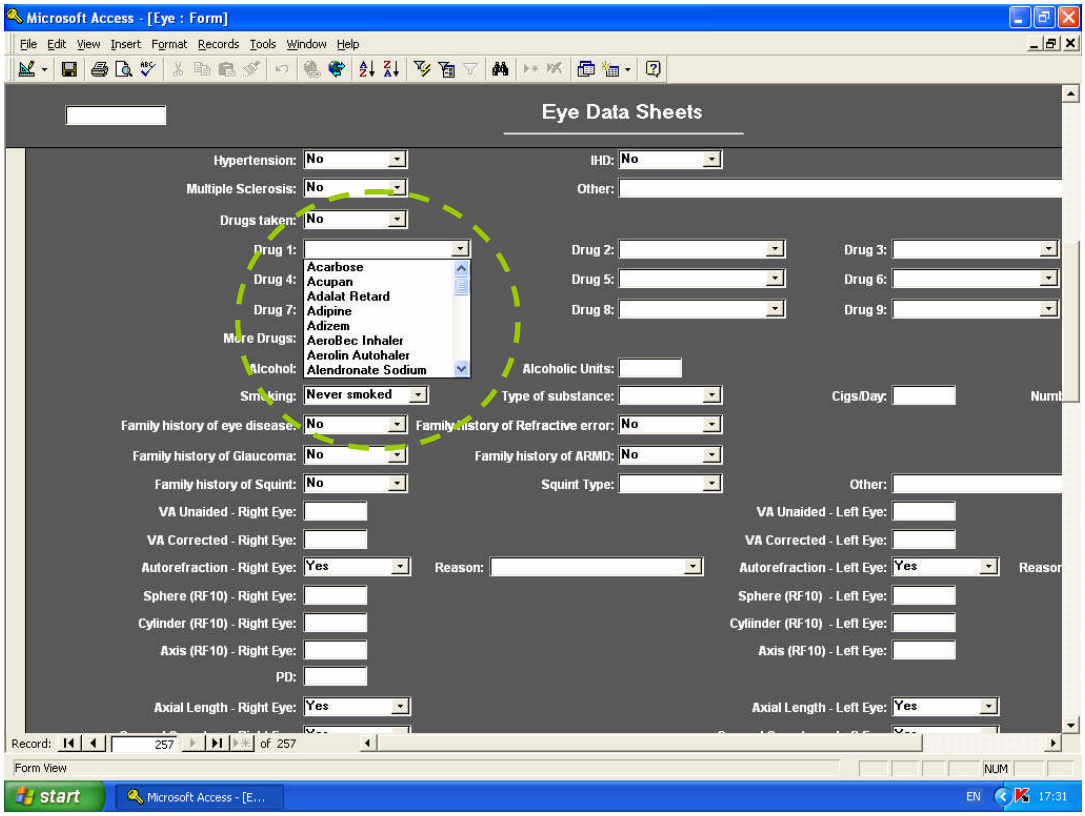


FIGURE 4.29: ORCADES EYE DATABASE –DROP-DOWN MENU FOR MEDICATION

Each field had a variety of checks to reduce data entry errors. For example, all date fields had to be entered in the format dd/mm/yyyy. An error message was generated if a date did not conform to this format, (for example, month=13), or a date out with the specified range was entered. For example, if the date of birth of an individual was entered as 20/05/2007 an error message would be generated. Numerical fields had range checks and an error message was generated if the entry was out of this range. A mean and standard deviation for each numerical value was calculated for interval data. After all data had been entered into the database, a random 10% of the datasheets were re-entered and compared to the initial entries to check the accuracy of data entry. No

numerical discrepancies were found and less than 1% qualitative differences (for example of a capital letter instead of a simple when typing in free text.).

4.3 DATA ANALYSIS

4.3.1 INTER-OBSERVER VARIATION

Inter-observer variation was completed in June 2008 with Dr. Brian Fleck, Consultant Ophthalmologist at the Princess Alexander Eye Pavilion, Edinburgh for the quantitative traits of central corneal thickness, axial length, corneal curvature, anterior chamber depth, white-on-white and intraocular pressure. The measurement of refraction is automated so there is little element of observer variation. Volunteers who lived on the Mainland and had no problems with mobility were invited to attend inter-observer variation clinics at the Orcades Study Centre. At the eye clinic, each volunteer had the above QTs measured as previously described by VKKK and BWF. The order of examiners alternated and both were blind to previous results and to each others findings. Data was entered into a separate database and intra-class correlation coefficients calculated using SPSS 18 (IBM, Chicago, Ill., USA). The calculation of optic nerve head parameters is dependent on the contour line which is drawn by the examiner around the optic nerve head or around PPA. Other parameters such as cup disc ratio are calculated automatically but are dependent on the correct placing of this line. 10% of optic nerves and all PPA had contour lines re-drawn by Mr. E.White, Glaucoma Research Institute,

Moorfields Eye Hospital, City Road, London. Intra-class correlation coefficients were calculated for disc area and PPA area. Results for inter-observar variation are presented in chapter 5, section 5.2.

4.3.2 MULTIVARIABLE ANALYSIS

Descriptive statistics and multiple regression to investigate the relationship between the different variables was carried out using Minitab 14 (Minitab Inc., State College, PA, USA). Intra-class correlation coefficients for inter-observer variation analysis were generated using SPSS 18 (IBM, Chicago, Ill., USA). As the Orcades Study is a family based study with potentially high levels of relatedness between participants, it violates one of the assumptions of multiple regression techniques – the assumption of independence between observations. The common thread that unites all correlated studies is that observations are clustered, and individuals or observations within the same cluster are likely to have similar outcomes compared to observations between clusters. For example, measurements within the same family are more likely to be similar due to shared genetic and environmental factors than between families. This within cluster correlation needs to be accounted for before the multivariate analysis otherwise the statistical significance of the results may be inflated. Therefore prior to carrying out multiple regression, residuals were generated using taking into account the relatedness of individuals. This work was carried out by Dr. Veronique Vitart (MRC Human Genetics Unit, Edinburgh) one of the members of the team with access to the

genetic information in the study. In brief, using GenABEL software (Aulchenko et al., 2007) to take into account the structure of the sample, residuals for the required quantitative traits were generated taking into account the relatedness of the individuals using whole genome genotypes, with age and sex fitted as fixed effects. The rnktransform function of GenABEL was used on all traits to normalize their distribution. The residuals generated were then used in multiple regression models to investigate the relationship between these quantitative traits using Minitab 14 (Minitab Inc., State College, Pennsylvania, USA). The volume of quantitative trait data generated by this study is enormous. In addition to data on intraocular pressure, central corneal thickness, axial length, anterior chamber depth, axial length, corneal curvature, white-to-white, refractive error, over 30 quantitative traits are generated by the HRT III for the optic nerve and surrounding peripapillary area. Because of a combination of time pressure and limited manpower, we had to limit the number of traits we had analyzed to take into account relatedness. Traits were chosen on the basis of biological importance and credibility, and association with primary open angle glaucoma. In addition, we also limited the analysis to one eye.

Information regarding both eyes was available for the majority of patients. To analyse both or one eye, and if one eye, which eye should be anlaysed, is a common quandry in ophthalmological research (Glynn & Rossner 1992, Murdoch et al., 1998). As both genetic and global, though not local environmental factors are similar in both eyes, theoretically, if we accept a complex model of disease and development, one eye is likely to resemble the other. Ocular diseases however, can be unilateral. This could be

because the disease is rare. For example, in 99% of cases, choroidal melanoma tends to be unilateral (Murdoch et al., 1998). Or enviromental exposure is unilateral, for example, in herpes simplex virus infection. In other diseases such as POAG, the cause for unilateral disease is unclear. However, if unilateral, neither left nor right eye tends to be favoured in ocular disease (Murdoch et al., 1998). The advantage of using a single eye, is less complex statistical techniques can be utilized to analyse data. However, by using only one eye information is lost, reducing the potential power of the study. Furthermore, there is a potenial that bias could be introduced by the selection of one eye over another. In cases where one eye is effected, for example, choroidal melanoam or in unilateral glaucoma, the effected eye or eye with more extreme value should be investigated.

The great advantage of using information from both eyes is it increases power. Two approaches have been used, the first is the pooling or averaging of data, which also leads to the loss of information, though some would argue less information is lost compared to using one eye (Glynn & Rossner 1992, Murdoch et al., 1998). Loss of information would be particularly great if the correlation between the two eyes is poor. The second involves using information from both eyes separately. However, in this scenario, correlation between the eyes need to be accounted for by using more advanced statistical techniques such as generalized estimating equations. Not accounting for the correlation between eyes will mean the final conclusions that are reached will be misleading, estimates will be more precise and p values lower.

Ideally we would have chosen both eyes from each participant and used statistical techniques to account for the correlation in a manner analagous to accounting for relatedness. However, for pragmatic reasons, and because there is little evidence in the literature and in our own experience of laterality in ocular disease (Murdoch et al., 1998) we limited the analysis to one eye. As discussed before, genetic information was available to few members of the group and hence manpower was limited. The right eye was chosen randomly. Subsequent analysis demonstrated (see results section), in the majority of traits, there was no significant difference between eyes. Even if the difference was statistically significant, it was not clinically significant. This differentiation between clinical and statistical signficant is made throughout the results section. This is because significance testing assesses whether any noted differencs between groups are due to chance, the p value signifying the probability of obtaining the given value or one more extreme if the null hypothesis is true (Petrie & Sabin 2005). Even if there is a statistically significant difference does not necessarily mean that this difference is important. In the context of this study, quantitative traits can only be measured with accuracy to a certain degree. Any difference below this level is difficult to interprate. For example, imagine the mean difference between intra-ocular pressure in right and left eyes is 0.3mmHg and this difference is found to be statistically significant. However, with Goldman applanation tonometry IOP can only be measured, at the greatest, with any kind of accuracy to 1mmHg, and some would argue, only to 2mmHg as gradations on the machine are 2mmHg apart. Hence effect size is too small to be meaningful in a clinical setting.

4.4 SUMMARY

Preliminary data for the Orcades Eye Study was collected between April 2007 and November 2007. Data was collected at the Orcades Centre, Victoria Street, Kirkwall Orkney. Each appointment lasted on average 45 minutes, when the project was explained, informed consent obtained, a brief medical and ocular history obtained and data for a number of ocular biometric traits collected. This included refractive error measured with a Canon-RF10 autorefractor, axial length, corneal curvature, white-on-white measured by the Carl Zeiss IOLmaster, corneal thickness measured with the IOPac Pachymeter and a number of optic nerve and peripapillary parameters using the Heidelberg Retinal Tomograph III. In addition, volunteers underwent a qualitative ocular assessment and had their intraocular pressure assessed using Goldman Applanation Tonometry. At the end of the appointment volunteers were provided with feedback regarding their ocular state, and referral letters sent to GPs as required. The data from this examination was entered into an Access 7 database, entry validated and data analyzed using mainly Minitab 14 Software. Optic nerve head parameters were initially analyzed using HRT III software, converted into an Excel spreadsheet then analyzed using Minitab 14. The Orcades Eye Study is a family based cross sectional study. Hence, traits had relatedness between individuals taken into account before the final analysis.

CHAPTER 5

- RESULTS -

OVERALL CHARACTERISTICS OF STUDY SAMPLE

5.1 OVERVIEW

The following results are based on a total of 256 individuals who accepted the invitation to participate in the Orcades Eye Study and eventually attended. 635 individuals were invited. 257 initially accepted but one failed to complete the study.

Eyes which had undergone intraocular surgery were excluded from further quantitative analysis except in the analysis of visual acuity (n=7, 5 phacoemulsifications, 1 retinal detachment surgery, 1 phacoemulsification followed by subsequent retinal detachment surgery). The following eyes were also excluded from further analysis except in the analysis of visual acuity: The anterior segment of eyes which had undergone refractive surgery (n=2); of an individual with granular corneal dystrophy; the eyes of a volunteer with a form of congenital optic atrophy with a visual acuity of hand movements and poor fixation; the eyes of a volunteer with severe osteoarthritis who found maintaining the correct position for the use of some of the equipment difficult; the eyes of volunteers with previous orbital cancer (n=2). 247 inidviduals participated in the univariate analysis. By the time this data was ready for analysis, still only a limited

number of individuals had been genotyped, so this number was reduced further to 175. diagram 5.1 below summarizes the flow of participants in the study.

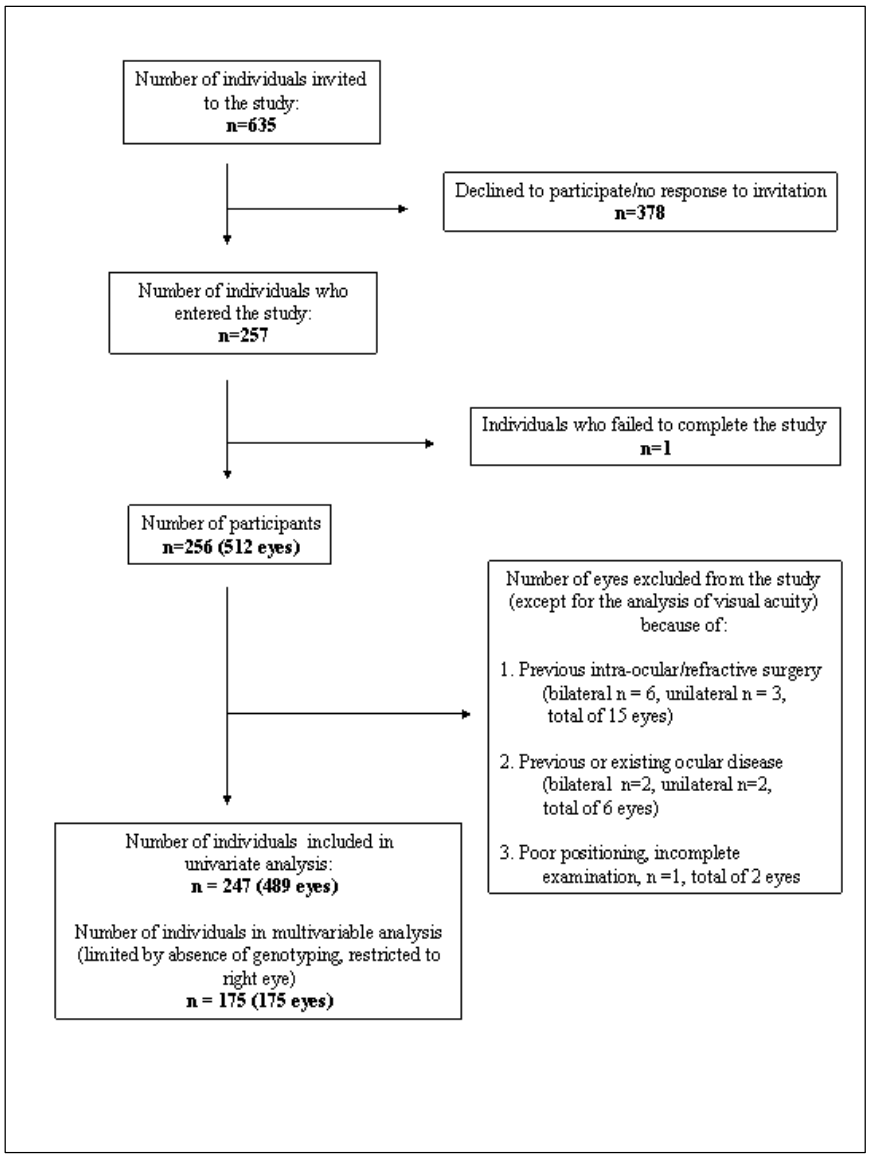


FIGURE 5.1: PARTICIPANT FLOWCHART

The age distribution of the sample is shown in figure 5.2 below.

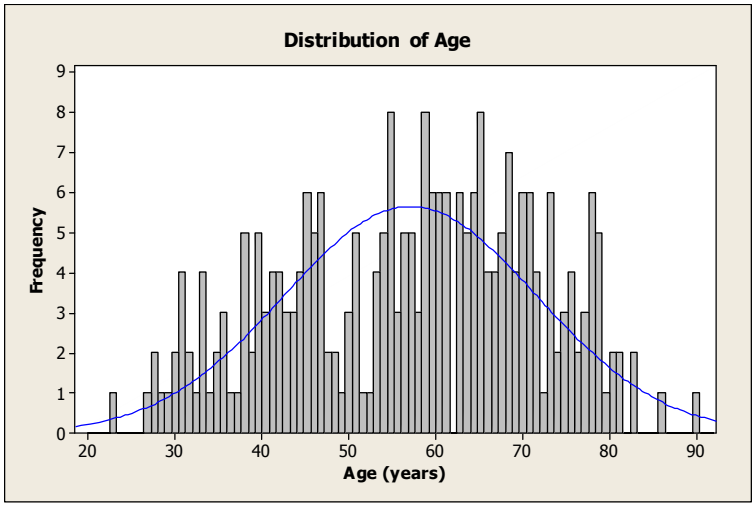


FIGURE 5.2: AGE DISTRIBUTION OF THE STUDY POPULATION

Individuals in our sample had a mean age of 58 years with a standard deviation of 14 years to the nearest year, a minimum age of 23 years and a maximum age of 90 years on the day of attendance. 37.9% of the cohort were male with a mean age of 58 years (standard deviation 15 years). 62.1% were female with a mean age of 56 years (standard deviation of 14 years). Table 5.1 shows the demographic characteristics of our sample compared to the Orkney population, the Scottish population and the population of the United Kingdom.

TABLE 5.1: CHARACTERISTICS OF THE STUDY POPULATION COMPARED WITH THE POPULATION OF ORKNEY, SCOTLAND AND THE UNITED KINGDOM.

POPULATION	STUDY PARTICIPANTS	ORKNEY*	SCOTLAND*	UNITED KINGDOM*
Age Group				
16 - 29 years	2.7%	13.7%	17.5%	17.6%
30 - 44 years	21.1%	22.1%	23.0%	22.6%
45 - 59 years	30.5%	21.5%	19.3%	18.9%
60 - 74 years	34.0%	15%	14.0%	13.3%
75+	11.7%	7.8%	7.1%	7.5%
Gender				
Male	37.9%	49.3%	48%	48.6%
Female	62.1%	50.6%	52%	51.4%
Total	256	19,245	5,062,011	58,789,194

* Census data, April 2001.

The past ocular, medical and drug histories of the study population are summarized in tables 5.2, 5.3 and 5.4.

TABLE 5.2: SELF REPORTED PAST OCULAR HISTORY

PAST OCULAR HISTORY	NUMBER OF INDIVIDUALS n = 256 (Percentage of Total Study Population)
Refractive Error	41 (16.0%)
Amblyopia	16 (6.2%)
Squint	6 (2.3%)
Glaucoma	0
Ocular Hypertension	1 (0.4%)
OTHER	
Bilateral granular dystrophy	1 (0.4%)
Congenital bilateral optic atrophy	1 (0.4%)
Familial optic atrophy	1 (0.4%)
Von Hippel Lindau	1 (0.4%)
Orbital cancer	2 (0.8%)
Red/green colour blindness	1 (0.4%)
Horner's Syndrome	2 (0.8%)
SURGERY	
Cataract Surgery	
Unilateral	2 (0.8%)
Bilateral	4 (1.6%)
Retinal Detachment Surgery	2 (0.8%)
Laser for retinal detachment/tear	2 (0.8%)
PRP for VHL	1 (0.4%)
Refractive surgery	2 (0.8%)
Squint Surgery	3 (1.2%)
Ectropian correction	1 (0.4%)

TABLE 5.3: SELF REPORTED PAST MEDICAL HISTORY

PAST MEDICAL HISTORY	NUMBER OF INDIVIDUALS n = 256 (Percentage of Total Study Population)
CARDIOVASCULAR DISEASE	
Hypertension	61 (23.7%)
Hypotension	1 (0.4%)
Coronary Heart Disease (Angina, previous MI, history of previous CABG)	20 (7.7%)
Hypercholesterolemia	17 (6.6%)
Heart Disease Other (Disorders of heart rate, rhythm and conduction, valve disease)	14 (5.4%)
Previous DVT	1 (0.4%)
Previous TIAs, CVAs	3 (1.2%)
RESPIRATORY DISEASE	
Asthma	15 (5.8%)
Asthma/ COPD	1 (0.4%)
Sarcoid of the lungs (and legs)	1 (0.4%)
Wegener's Granulomatosis	1 (0.4%)
ENDOCRINE	
Diabetes Type I	2 (0.8%)
Diabetes Type II	3 (1.2%)
Hypothyroid	22 (8.6%)
NEUROLOGICAL DISEASE	
Multiple Sclerosis	2 (0.8%)
Parkinson's Disease	1 (0.4%)
Subarrachnoid Haemorrhage	1 (0.4%)
Migraines	2 (0.8%)
GASTRO-INTESTINAL DISEASE	
Gastro-oesophageal reflux disease/hiatus hernia/peptic ulcer	9 (3.5%)
Diseases of the gall bladder, liver and pancreas	4 (1.6%)
Inflammatory Bowel Disease, diverticulitis, irritable bowel syndrome, constipation	9 (3.5%)

TABLE 5.3 (CONTINUED): SELF REPORTED PAST MEDICAL HISTORY

PAST MEDICAL HISTORY	NUMBER OF INDIVIDUALS n = 256 (Percentage of Total Study Population)
DISORDERS OF THE BONES AND JOINTS	
Osteoarthritis	7 (2.8%)
Rheumatoid Arthritis	4 (1.6)
Ankylosing Spondylitis	1 (0.4%)
Gout	1 (0.4%)
Arthritides of unknown origin, other non-specific "joint problems"	12 (4.7%)
Osteoporosis	8 (6.7%)
OTHER	
Disorders of the ear, nose or throat	3 (1.2%)
Disorders of the skin	3 (1.2%)
Genitourinary/renal	3 (1.2%)
Atopy (hayfever)	3 (1.2%)
Psychiatric (depression)	7 (2.7%)
Previous operations (varicose vein, appendicectomy, cholecystectomy, hysterectomy, neck lumpectomy)	5 (1.9%)
Cancer	
Bone	1 (0.4%)
Breast	2 (0.8%)
Prostate	1 (0.4%)

TABLE 5.4: SUMMARY OF SELF REPORTED DRUG HISTORY

DRUG HISTORY	NUMBER OF INDIVIDUALS n = 256 (Percentage of Total Study Population)
No medication	104 (41.1%)
Medication for cardiovascular diseases (including antihypertensives, antiplatelet drugs, lipid level lowering agents and diuretics)	76 (30%)
Medication for pulmonary diseases (including bronchodilatators and antihistamines)	22 (8.7%)
Medication for metabolic diseases (including drugs for diabetes and thyroid disease)	26 (10.3%)
Medication for gastrointestinal diseases (including antacids, H_2 blockers and proton pump inhibitors)	29 (11.5%)
Medication for psychiatric or neurological disorders (including antidepressants)	18 (7.1%)
Other (including oral contraceptives, NSAIDs, vitamins)	25 (9.9%)

44.0% of the study population claimed to have smoked cigarettes at some point in their life, having smoked for a mean of 15 pack years (95% confidence interval 12 to 18 pack years). Only 11% of this 44% however, (approximately 5% of the total study population) were current smokers. Other details of regarding smoking in the study population are summarized in the table 5.5 and compared to data from the populations of Scotland and the United Kingdom.

TABLE 5.5: SMOKING HISTORY OF THE STUDY POPULATION COMPARED WITH THE ADULT POPULATION OF SCOTLAND AND THE UNITED KINGDOM

POPULATION		STUDY POPULATION			SCOTLAND*			UNITED KINGDOM**		
		Female	Male	All	Female	Male	All	Female	Male	All
CURRENT SMOKERS	Cigarette	6%	3%	5%	25%	27%	26%	21%	22%	21%
	Cigar	<1%	0	1%	-	-	-	<1%	2%	1%
	Pipe	0	0	0	-	-	-	<1%	1%	<1%
	Other	<1%	0	<1%	-	-	-	-	-	-
EX-REGULAR SMOKERS	Cigarette	36%	45%	39%	19%	19%	19%	22%	30%	25%
	Cigar	0	2%	<1%	-	-	-	-	-	-
	Pipe	0	2%	<1%	-	-	-	-	-	-

* The Scottish Government: Scottish Health Survey 2008
****** Office for National Statistics: General Lifestyle Survey 2008, Smoking and Drinking Among Adults, 2008

81% of the total group admitted to having drunk alcohol in the past year. Of these approximately a third (30%) claimed to be only "occasional drinkers" (defined as less than 1 unit a week) with the remaining 70% claiming to have drunk at least 1 unit per week. 19% claimed to be teetotal. Mean alcohol consumption amongst the regular drinkers was around 8.5 units a week (95% confidence interval 7 to 10 units).

TABLE 5.6: ALCOHOL CONSUMPTION OF STUDY POPULATION COMPARED WITH THE ADULT POPULATION OF SCOTLAND AND THE UNITED KINGDOM

POPULATION	STUDY POPULATION			SCOTLAND*			UNITED KINGDOM**		
	Female	Male	All	Female	Male	All	Female	Male	All
Non-drinker	24%	10%	19%	13%	10%	-	17%	11%	14%
Occasional drinker (<1 unit/week)	28%	20%	25%	18%	8%	-	-	-	-
Regular drinker (>1 unit/week)	48%	70%	56%	69%	82%	-	55%	70%	-
Average weekly alcohol consumption of regular drinkers (units)	7.2	9.9	8.5	8.6	18	-	8.4	16.6	12.2

***** The Scottish Government: Scottish Health Survey 2008
****** Office for National Statistics: General Lifestyle Survey 2008, Smoking and Drinking Among Adults, 2008

Figures 5.3, 5.4 and tables 5.7, 5.8 summarize the distribution of unaided and corrected visual acuity respectively in right and left eyes.

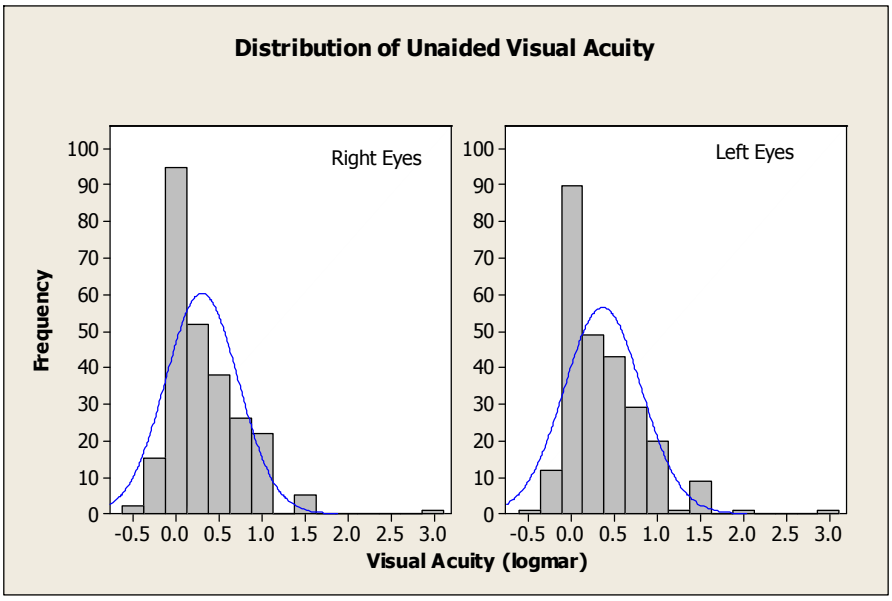


FIGURE 5.3: DISTRIBUTION OF UNAIDED VISUAL ACUITY

TABLE 5.7: SUMMARY STATISTICS FOR UNAIDED VISUAL ACUITY

Unaided Visual Acuity	Right Eye logMAR	Left Eye LogMAR
All Participants		
Mean	0.31	0.36
95% Confidence Interval for Mean	0.26-0.36	0.30-0.42
Standard Deviation	0.42	0.45
Range	-0.38 – 3.00	-0.38 – 3.00
Mode	0	0
Median	0.2	0.2
Inter-quartile Range	0 – 0.58	0 – 0.6
Males Mean (95% Confidence interval)	0.27(±0.07)	0.35(±0.09)
Females Mean (95% Confidence interval)	0.33(±0.07)	0.36(±0.07)

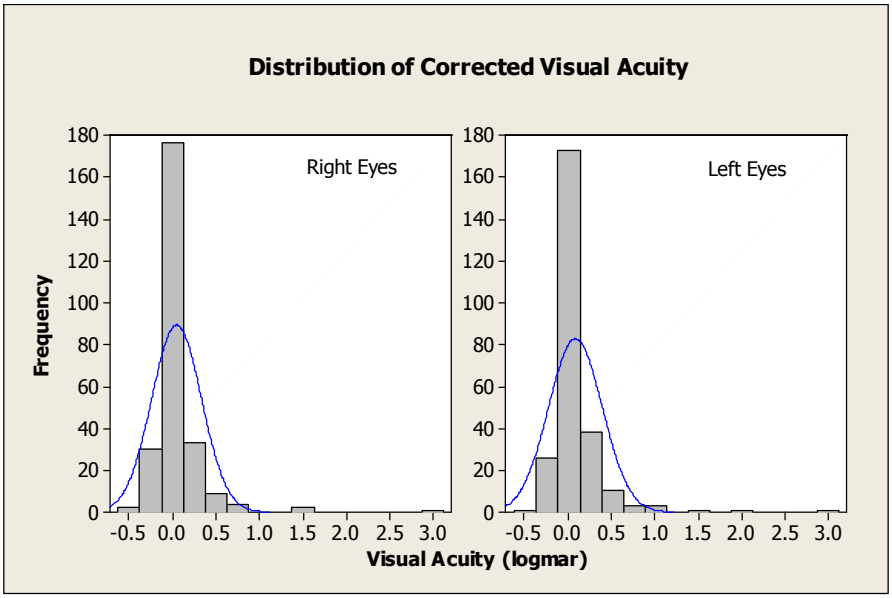


FIGURE 5.4: DISTRIBUTION OF CORRECTED VISUAL ACUITY

TABLE 5.8: SUMMARY STATISTICS FOR CORRECTED VISUAL ACUITY

Corrected Visual Acuity	Right Eye logMAR	Left Eye LogMAR
Mean	0.05	0.07
95% Confidence Interval for Mean	±0.04	±0.04
Standard Deviation	0.29	0.31
Range	-0.38 – 3.00	-0.38 – 3.00
Mode	0	0
Median	0	0
Inter-quartile Range	-0.10 – 0.10	-0.10 – 0.10
Males Mean (95% Confidence interval)	0.03(±0.05)	0.08(±0.07)
Females Mean (95% Confidence interval)	0.06(±0.05)	0.07(±0.05)

Descriptive statistics for uncorrected and corrected visual acuity are reported in tables 5.7 and 5.8 respectively. 64% of study participants had an uncorrected logmar VA of 0.3 (Snellen equivalent of 6/12) or above in their right eye, and 58.4% in their left eye. 45.1% of participants had an uncorrected logmar VA of 0.18 (6/9 Snellen equivalent) in their right eye and 41.6% in their left eye. 35.3% of participants had an uncorrected logmar VA of 0 or greater in their right eye and 31.4% in their left eyes. The differences in visual acuity between men and women, for both corrected and uncorrected visual acuity, were not statistically significant at the 5% level (all p values greater than 0.05). Two volunteers met the International Classification of Diseases 10th Edition (ICD-10) criteria for visual impairment - "a visual acuity less than 6/18 in the better eye with the best possible correction." Both had been diagnosed with forms of optic atrophy.

5.2 INTER-OBSERVER VARIABILITY

Intra-class correlation coefficients ranged from 0.858 (95% confidence interval 0.693-0.935) for intra-ocular pressure in the right eye to 1.000 (95% confidence interval 1.000-1.000) for left axial length. Table 5.9 summarizes values for inter-observer variation, and possible reasons for these results discussed in section 5.3.

TABLE 5.9: SUMMARY OF INTER-OBSERVER VARIABILITY FOR OCULAR QUANTITATIVE TRAITS

Quantitative Trait	Side	Intraclass Correlation Coefficient	95% Confidence Intervall
Average IOP	Right	0.858	0.693-0.935
	Left	0.887	0.755-0.948
Central corneal thickness	Right	0.988	0.969-0.995
	Left	0.992	0.979-0.997
Average axial length	Right	0.975	0.945-0.988
	Left	1.000	1.000-1.000
Average K1	Right	0.998	0.996-0.999
	Left	0.999	0.999-1.000
Average K2	Right	0.997	0.994-0.999
	Left	0.997	0.993-0.999
Average R1	Right	0.998	0.996-0.999
	Left	0.999	0.999-1.000
Average R2	Right	0.997	0.994-0.999
	Left	0.999	0.997-0.999
Average ACD	Right	0.996	0.992-0.998
	Left	0.985	0.967-0.993
Average W	Right	0.980	0.955-0.991
	Left	0.979	0.951-0.991
ONH parameters	Right/Left	0.977	0.967-0.984
PPA	Right/Left	0.958	0.940-0.971

5.3 DISCUSSION

Establishing the Orcades Eye Project was a challenging endeavor. The location of the Orcades Study Centre was ideal. Situated at the centre of Orkney's commercial district, with excellent transport links, it was easily accessible to Mainland Orcadians and Islanders alike. Following the initial pilot study, the new data collection sheet and

amended protocol worked well, and once the data base was tested, problems sorted out, this too worked extremely well.

The initial analysis of this data is an epidemiological one. In general, the nature of empirical research is such, that most conclusions are derived from incomplete observations, or samples. The ideal strategy for investigating quantitative traits related to primary open angle glaucoma would be to gather data from the entire Orcadian population. Complete ascertainment strategies have been pursued by a number of studies such as the Framingham Study in New England (Kahn et al., 1977a). Alternatively, samples representative of the population can be sought, using some form of randomization strategy (Kalsbeek and Heiss, 2000). Recorded attempts to estimate population parameters using sampling strategies date back to the 18th century when efforts were made to infer the population of France (Kalsbeek and Heiss, 2000). The use of randomization strategies emerged in the 19th century, initially with some controversy surrounding them, but then gradually gaining credence in the early part of the 20th century (Stephan, 1948). Sampling has it's advantages. It reduces the complexity and cost of collecting data which is especially relevant if the population under study is large, dispersed or resources are limited. Manpower and effort can be concentrated on a smaller group with the potential to increase quality at the expense of quantity if resources are limited. However, sampling can introduce sampling error. Despite using strategies to limit bias, the sample may not be representative of the population in question. The long-term aim of this project was to establish a population isolate study investigating the inheritance of quantitative traits related to primary open angle

glaucoma, with the objective of finding regions of the genome and genes associated with its susceptibility. As we utilized volunteers and resources of the already established cross-sectional family based genetics study the Orcades Cardiovascular Disease Study, to establish this project, our recruitment strategy had to be in line with the aims, objectives and long term strategy of the overall Orcades project. We also had limited resources and time. These factors constrained what methods we could employ to recruit volunteers. Volunteers who had been the first to participate in the cardiovascular and anthropometric arms of the Orcades study, were given the opportunity to be the first to participate in the ocular arms of the Orcades study. These were volunteers recruited from the West Mainland and other Orcadian Islands. The reason for this was two fold. First it was to prevent volunteer fatigue – volunteers had undergone a battery of investigations for the cardiovascular and anthropometric arms of the project, so we attempted to leave as much time between visits as possible. The second reason was these volunteers were more likely to have genome-wide scans completed at the end of the data collection period of the PhD. This method of ascertainment means our sample may not be reflective of the rest of the indigenous population of Orkney. A comparison of age distributions between our sample and a number of populations (table 5.1) shows that our study sample has a lower percentage of participants in the 16-29 age group, a higher number of participants who are 60 years and older and a higher proportion of females taking part compared to the population distribution of age and gender in the populations of Orkney, Scotland and the United Kingdom. Fewer participants in our study were current smokers (5% versus 21%), and more participants claimed to be teetotal (19% versus 14%) compared to national averages (table 5.5 and table 5.6). A wide range of

medical and ocular problems were reported by our cohort (tables 5.2 and 5.3). The most common self reported medical condition was hypertension (23.7% of the study population). The prevalence hypertension in our sample was slightly lower than the prevalence of hypertension reported in the Scottish Health Survey as well as the Health Survey for England (approximately 33% of men and 31.7% of women in Scotland over the age of 16 in 2003, approximately 31% for men and 28% for women in England in 2005 (Anon, 2006a)).

Visual acuity in our sample ranged from -0.38 to 3.00 logMAR, with 64% of participants having an uncorrected logMAR acuity of greater than 0.3. Two volunteers met the criteria for visual impairment as defined by ICD-10. Both individuals had been diagnosed with forms of primary optic atrophy. The term "optic atrophy" describes the morphology of the optic nerve following a disease process that involves the death of the retinal ganglion cells and their axons (Sadun, 2008). Subjectively individuals have varying levels of visual dysfunction depending on the nature of damage. The term primary optic atrophy encompasses a wide variety of pathologies, united by a common morphological appearance - disc pallor with loss of optic nerve fibers but otherwise little disruption to the architecture of the optic nerve head. Causes of primary optic atrophy are varied and range from hereditary forms to ischemic, inflammatory and neuro-ophthalmic insults. One volunteer had been diagnosed as having "congenital optic atrophy" and the second, as having "familial" optic atrophy. The prevalence of visual impairment or blindness secondary to optic atrophy from population based surveys has been found to be between 0.04 to 0.8% (Munoz et al., 2000, Tielsch et al., 1995a), which

is not out with our finding of 0.78%. The prevalence of visual impairment in the United Kingdom has been reviewed recently by Tate et al. in a report commissioned by the Royal National Institute for Blind People (Tate et al., 2005) Overall, the leading causes of visual impairment in Scotland and in the United Kingdom are age-related macular degeneration, glaucoma and diabetic retinopathy (Bamashmus et al., 2004, Tate et al., 2005), in contrast to our study. It is worth noting that though these findings are interesting and help paint a picture of our study sample, the sample size is too small to draw any meaningful conclusions or extrapolate to make generalizations regarding visual impairment in the Orcadian population.

Intra-class correlation coefficients ranged from a low of 0.858 (95% confidence interval 0.693-0.935) for intra-ocular pressure in the right eye to a high of 1.000 (95% confidence interval 1.000-1.000) for left axial length. The former value with wide confidence intervals probably reflects the nature of intraocular pressure, as well the nature of it's measurement (Whitacre and Stein, 1993). Because a number of values for ICC are very high, we re-examined the data and analysis but came to the same conclusions.Such high levels of agreement have been previously reported in the literature in other studies using partial coherence inferometry to measure ocular biometric traits (Sacu et al., 2005, Wong et al., 2005, Shirayama et al., 2009) We believe this value is a reflection on the reliability and reproducibility of the IOLmaster.

We had aimed to collect data on quantitative traits related to primary open angle glaucoma from a minimum of 1000 volunteers. Unfortunately a number of problems

which we hadn't anticipated, delayed the start and interfered with the progress of this project. The initial date was delayed as negotiations with the vendor, and the legal formalities required to procure the premises and carry out the required renovations were protracted. The renovations were delayed further due to the paucity of appropriate manpower and halted for a short period due to inclement weather. Several bouts of equipment failure and staffing issues slowed/arrested data collection once it commenced. At the start of the project, there were also problems with volunteer non-attendance. Once a reminder "courtesy phone call" to volunteers a day or two before their appointment was instituted, this non-attendance declined. Overall, we were able to collect quantitative trait data from 256 volunteers. In retrospect, our original plans – to secure funding, find and renovate premises, source appropriate equipment, train in the use of that equipment, establish and refine standard operating procedures, set up, refine and verify databases, recruit volunteers, then gather both quantitative and qualitative data from 1000 volunteers, enter and analyze this data within the time frame of a PhD, was unrealistic. However, the data that has been collected is of excellent quality. Once initial problems had been solved, the data collection and entry procedures and database all worked incredibly well. Though my role in data collection has now come to a close, there are plans to continue to collect further POAG related quantitative trait data in Orkney for a meaningful genetic analysis. To date, the only other population based study which has collected such a wide range of POAG associated quantitative traits including peripapillary atrophy and central corneal thickness is the Beijing Eye Study (Jonas et al., 2009).

CHAPTER 6

- RESULTS -

CENTRAL CORNEAL THICKNESS AND CORNEAL DIAMETER IN THE SCOTTISH POPULATION ISOLATE OF ORKNEY

6.1 RESULTS

The distribution of corneal thickness and width are shown in figures 6.1 and 6.2, and summary statistics reported in tables 6.1 to 6.4. Mean CCT in both eyes was 540µm (95% confidence interval 536-544µm). Bivariate analysis suggested a highly significant relationship between IOP and central corneal thickness (p=0.004) which was confirmed on multivariate analysis (see table 6.2). We found no statistically significant relationship between CCT and other ocular biometric parameters that were assessed including axial length and optic nerve parameters. We also found no relationship between CCT and age or gender (p = 0.124, p = 0.260 respectively on multivariate analysis).

Mean corneal diameter in our sample was 12.3mm in the right eye and 12.2mm in the left eye (see table 6.3). The difference between the corneal diameters of right and left eyes (0.0503mm) was statistically significant (paired t-test, p = 0.000) but not clinically significant (95% confidence interval for mean difference 0.024-0.077mm). Neither age

nor gender had an effect on corneal diameter. The only ocular biometric parameter to have a statistically significant relationship with corneal diameter was corneal curvature ($p = 0.000$).

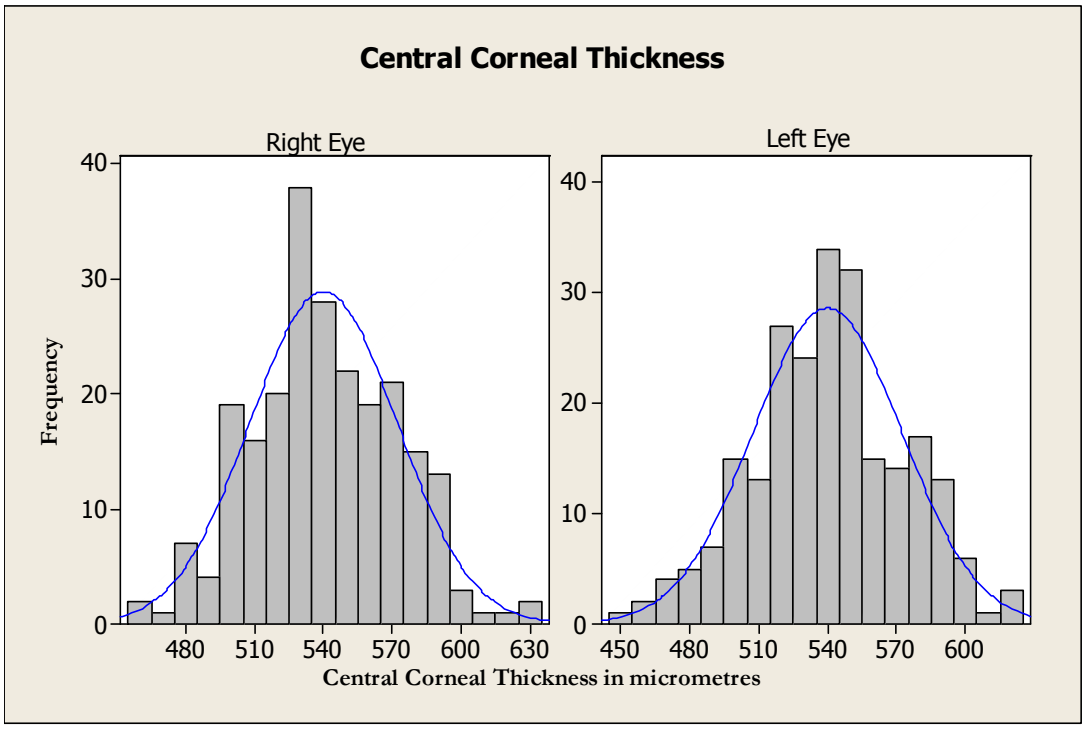


FIGURE 6.1: DISTRIBUTION OF CENTRAL CORNEAL THICKNESS

TABLE 6.1: SUMMARY STATISTICS FOR CENTRAL CORNEAL THICKNESS

	Right Eye (μm)	**Left Eye** (μm)
Mean	540	540
95% Confidence interval for mean	536-544	536-544
Standard Deviation	32	32
Range	455-629	453-624
Males Mean (95% confidence interval)	538 (532-544)	540 (534-546)
Females Mean (95% confidence interval)	541 (536-546)	539 (534-544)

TABLE 6.2: MULTIVARIABLE ANALYSIS FOR CENTRAL CORNEAL THICKNESS

COVARIATE	COEFFICIENT	STANDARD ERROR	P-VALUE
Age	-0.4064	0.262	0.124
Gender	-5.84	-5.85	0.260
Spherical Equivalent	0.032	0.087	0.714
Intraocular Pressure	1.2978	0.4479	0.004
Keratometry	228.0	119.6	0.059
Corneal Diameter	1.2113	0.8545	0.159
Anterior Chamber Depth	0.0162	0.0799	0.839
Axial Length	0.874	1.063	0.412
Nerve Fiber Layer Thickness	0.0799	0.0709	0.262
Rim Area	-0.0767	0.0836	0.361
Cup Area	0.0965	0.1553	0.535
Maximum Cup Depth	-10.99	21.37	0.608
PPA Area	-0.1244	0.2243	0.580
PPA peripheral extent	0.0936	0.1946	0.631

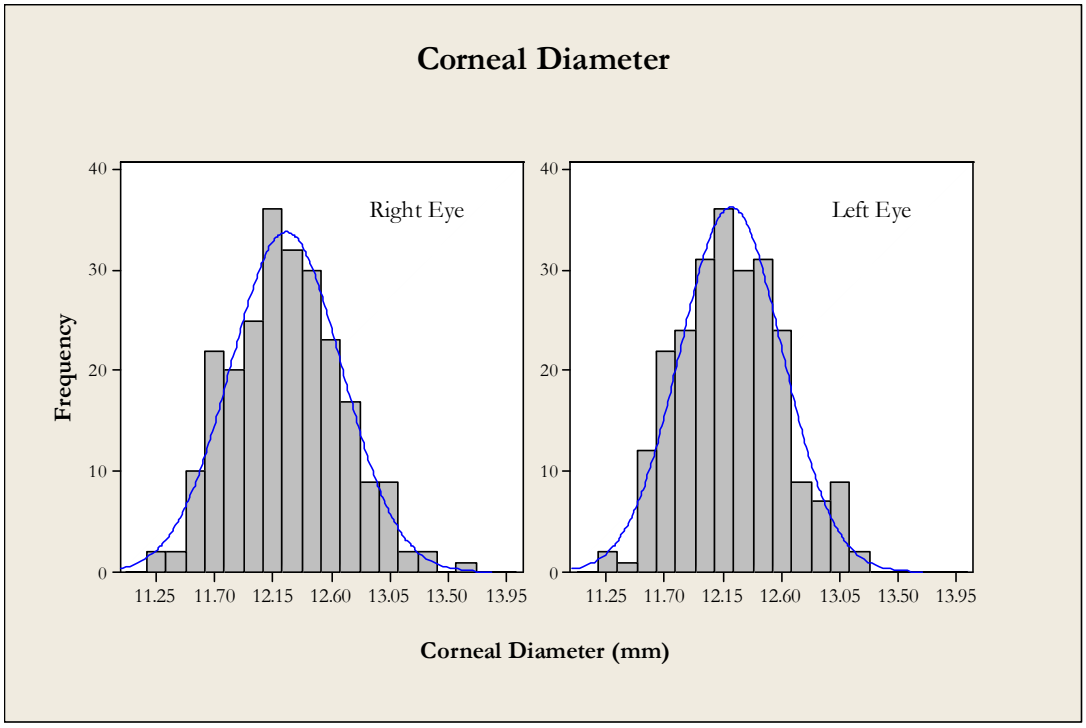


FIGURE 6.2: DISTRIBUTION OF CORNEAL DIAMETER

TABLE 6.3: SUMMARY STATISTICS FOR CORNEAL DIAMETER

	Right Eye (mm)	**Left Eye** (mm)
Mean	12.3	12.2
95% Confidence interval for mean	12.21 - 12.30	12.16 - 12.26
Standard Deviation	0.43	0.40
Range	11.3-13.7	11.3-13.2
Males Mean (95% confidence interval)	12.4 (11.5-13.3)	12.3 (11.5-13.1)
Females Mean (95% confidence interval)	12.2 (11.5-12.9)	12.1 (11.5-12.7)

TABLE 6.4: SUMMARY OF MULTIVARIABLE ANALYSIS FOR CORNEAL DIAMETER

COVARIATE	COEFFICIENT	STANDARD ERROR	P-VALUE
Age	-0.0004	0.0023	0.875
Gender	0.01578	0.0477	0.741
Spherical Equivalent	0.0113	0.0085	0.183
Intraocular Pressure	0.0114	0.0081	0.159
Keratometry	-80.539	9.649	0.000
Corneal Diameter	-0.0455	0.0446	0.310
Anterior Chamber Depth	0.0423	0.0069	0.000
Axial Length	-0.0321	0.1035	0.757
Nerve Fiber Layer Thickness	0.002	0.0069	0.775
Rim Area	-0.0147	0.008	0.069
Cup Area	-0.0047	0.0151	0.756
Maximum Cup Depth	-0.390	2.059	0.850
PPA Area	0.0172	0.0217	0.429
PPA peripheral extent	-0.0084	0.0188	0.656

6.2 DISCUSSION

Understanding corneal parameters has become increasing important in ophthalmology. The Ocular Hypertension Treatment Study and other studies identified CCT, as an independent risk factor for the progression of ocular hypertension to glaucoma (Gordon

et al., 2002, Medeiros et al., 2003a, Medeiros** et al., 2003, Medeiros*** et al., 2003, Miglior et al., 2007b). Though there is also some evidence which suggests that CCT is important as a risk factor in the progression of glaucoma (Kim and Chen, 2004) this is much disputed (Leske et al., 2003, Chauhan et al., 2005, Jonas et al., 2005a, Jonas et al., 2005b) (Congdon et al., 2006). Corneal thickness is not just important as a glaucoma related risk factor. In itself, it can be an indicator of corneal well being. In an age where increasing numbers undergo refractive surgery, normative values can aid the decision making process. For example, an intact CCT of 250μm is considered sufficient for the continuing mechanical stability of the cornea, but this number is based on a presumed "normal" corneal thickness of 550μm (Price et al., 1999, Wilkinson et al., 2008). An understanding of the thickness of the cornea is imperative (including it's relationship to other variables such as aging and gender) as this changes it's light scattering properties which are utilized in various forms of anterior segment photography (Wegener and Laser-Junga, 2009) and an understanding of anterior segment dimensions is invaluable for the design and use of phakic intraocular lenses (Hosny et al., 2000).

Table 6.5 summarizes mean CCT in populations across the globe.

TABLE 6.5: CENTRAL CORNEAL THICKNESS IN SELECTED POPULATIONS

STUDY	YEAR	POPULATION/ ETHNIC ORIGIN	AGE RANGE /MEAN AGE	METHOD	MEAN CCT ±SD
Greenland (Alsbirk)	1978	Inuit	7-89	Optical	523.7
Netherlands (Wolfs et al.)	1997	White Caucasian	≥55	Ultrasound	537.4 (95%CI: 533.8-540.9)
Mongolia (Foster et al.)	1998	Chinese	10-87	Optical Pachymeter	Right: 495±32 Left: 514± 32
Houston, Texas (La Rosa et al.)	2001	African Caribbean 49.7% White Caucasian 50.3%	65.8±10.5 63.3±12.5	Ultrasound	531.0 ±36.3 558.0± 34.5
(Doughty et al.)	2002	White Caucasian	32-60 61-82	Ultrasound	533 527
Reykjavik, Iceland (Eysteinsson et al.)	2002	White Caucasian	≥50	Scheimpflug	M 528 ±41 F 526 ±37 (no statistically significant difference
Barbados Eye Study (Nemesure et al.)	2003	African Caribbean 93% Mixed African Caribbean +White Caucasian 4% White Caucasian or other 3%	40-80	Ultrasound	529.8±37.7 537.8±34.0 545.2±45.7
Southern India (Vijaya et al.)	2005	Indian	40+	Ultrasound	505.93±31.11
Tajimi Eye Study (Suzuki et al.)	2005	Japanese	40+	Specular microscopy	517.5±29.8
Blue Mountains Eye Study (Healey et al.)	2005	White Caucasian	49+	Ultrasound	540±34
(Jonas and Holbach) Germany	2005	White Caucasian (Enucleated eyes)		Histomorphometric	616±108
Australia (Landers et al.)	2007	Indigenous Australians (Aborigines) White Caucasian Australians	51±14 SD 56±15 SD	Ultrasound	511 ± 34 541 ± 31

Australia (Durkin et al.)	2007	Indigenous Australians (Aborigines) White Caucasian Australians	44.8 ±14.5	Ultrasound	514.9 +/- 30.5 544.6 +/- 31.9
(Casson et al.)	2008	Burmese	≥40	Ultrasound	521.9±33.3
Beijing Eye Study (Zhang et al.)	2008	Chinese	45+		556.2±33.1
Chennai Glaucoma Study, (Vijaya et al.)	2008	Indian	40-103	Ultrasound	520.7
Turkey (Cankaya et al.)	2008	Turkish	40-59	Ultrasound	540±35
Singapore Malay Eye Study (Su et al.)	2009	Malay	40-80	Ultrasound	541.2
Ethiopia (Gelaw et al.)	2010	African	42.57±16.71	Ultrasound	518±32.92

Values for corneal thickness have shown a wide range in the literature from an average of around 500µm suggested by Goldman and Schmidt in the context of applanation tonometry to highs of 700 to 1000µm in pre 1950s reports (Doughty and Zaman, 2000). Current studies across the globe have demonstrated that central corneal thickness shows considerable inter-population variation, from a low mean of 495µm in a Mongolian population (Foster et al., 1998) to a mean of 558µm in self reported White Caucasians in Houston, Texas (La Rosa et al., 2001). Doughty and Zaman (Doughty and Zaman, 2000) calculated mean “normal” central corneal thickness to be 534µm (from 300 studies) or 536µm, with a standard deviation of 31µm (from a dataset of 230 where variance was specified). The mean CCT found in our study was 540µm, with a 95% confidence interval between 536-540µm. Taking into account the accuracy of pachymetry, these

findings are not dissimilar to the calculations of Doughty et al. (Doughty and Zaman, 2000) or the findings of other White Caucasian populations shown in table 6.5. The distribution of central corneal thickness in our sample was unimodal and we found no difference between the genders or any association with age.

The findings for a relationship between CCT and age and gender have been inconsistent, with some studies reporting associations, such as a decrease of CCT with age (Foster et al., 1998, Brandt et al., 2001, Nemesure et al., 2003) (Healey et al., 2005, Suzuki et al., 2005) and difference between genders (Alsbirk, 1978, Suzuki et al., 2005, Zhang et al., 2008) and many others finding little association with age or gender (Wolfs et al., 1997, Eysteinsson et al., 2002, Healey et al., 2005), (Gelaw et al., Zhang et al., 2008). In our study the only association we found between CCT and ocular parameters was a positive correlation between CCT and IOP. This association has been well described in many other studies (Wolfs et al., 1997, Foster et al., 1998, Eysteinsson et al., 2002, Suzuki et al., 2005, Doughty and Jonuscheit, 2007, Zhang et al., 2008, Su et al., 2009, Gelaw et al.). This has not been found to be the case in all populations however (Nemesure et al., 2003). Examining the IOP-CCT relationship has been a means of assessing the effect of CCT on IOP in a number of studies (Doughty and Zaman, 2000, Doughty et al., 2002) (Doughty and Jonuscheit, 2007). Overall, a thicker than “normal” cornea will overestimate intracameral IOP and a thinner than normal cornea will underestimate IOP (Doughty and Zaman, 2000). This finding has not been universal. The Barbados Eye Study for example did not find a statistically significant correlation between IOP and CCT in participants of African-Caribbean or “mixed” ancestry but did find this

correlation to be statistically significant in those of self reported White Caucasian ancestry, suggesting this relationship may show inter-population variation (Nemesure et al., 2003). This has clinical implications. In a population where CCT has little impact on IOP measurements, its measurement becomes less of a priority. Clinical time, resources and effort can be directed elsewhere. However, in a population such as ours where there is a relationship between CCT and IOP, pachymetry is of great importance in the assessment of an individual's IOP, especially in the diagnosis and management of glaucoma. Though many different guidelines suggest the measurement of central corneal thickness in the assessment of patients with primary open angle glaucoma, this is still not routine in clinical practice (European_Glaucoma_Society_II, 2003, American_Academy_of_Ophthalmology, 2008) .

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Multivariate analysis of the Ocular Hypertension Treatment Study dataset suggested that the effect of CCT on the progression of ocular hypertension to glaucoma was independent of it's effect on IOP (Gordon et al., 2002, Miglior et al., 2007b). The second explanation for this relationship between glaucoma and CCT, views central corneal thickness as a possible surrogate marker for some yet un-described biological risk factor such as some biomechanical aspect of the optic nerve head. During development. the corneal stroma and sclera share a common mesenchymal origin, and arise as one continuous coat (Moore and Persaud, 2007). Hence this hypothesis is grounded in reality though the evidence for any such an association is limited. Lesk et al found that patients with thinner corneas with ocular hypertension or open angle glaucoma showed greater compliance of their lamina cribrosa (measured by using optic cup depth as a surrogate)

than those with thicker corneas (Lesk et al., 2006) – a result which was not replicated by Nicoela et al (Nicolela et al., 2006). A number of other studies had reported associations between central corneal thickness and optic nerve head parameters when this project was being established (Iester and Mermoud, 2001), (Soans et al., 2004, Henderson et al., 2005, Pakravan et al., 2007). These studies were not population based and were carried out on individuals with glaucoma (Soans et al., 2004) or ocular hypertension (Henderson et al., 2005). Only Iester et al looked at normal eyes (n=44) (Iester and Mermoud, 2001). CCT measured by ultrasonic pachymetry was not correlated to retinal nerve fiber layer thickness measured by a confocal scanning laser polarimeter. At the time this project was being established, to our knowledge there had been no population based studies investigating the relationship between central corneal thickness and optic nerve head parameters. More recently a few reports have emerged. The Beijing Eye Study found a positive correlation between CCT measured by optic coherence tomography and optic disc area measured by planimetry of digitized optic disc photographs (Zhang et al., 2008). Thick corneas in this population were hence associated with larger optic discs. This contradicts the findings of Pakravan et al. who reported an inverse correlation between central corneal thickness and optic disc area but in patients with primary open angle glaucoma of mixed self reported ancestry (Pakravan et al., 2007). Similar findings to Pakravan et al. was reported in a healthy Turkish population. CCT was found to show a negative correlation to disc area, rim area, rim volume and retinal nerve fiber thickness (Cankaya et al., 2008). Gunvant et al. reported a correlation between optic rim area and CCT in 272 eyes from 144 patients of unreported ancestry from Norwich with normal tension glaucoma (n=50), ocular hypertension (n=48), primary open angle glaucoma

(n=71) and glaucoma suspects (n=103). A statistically significant positive correlations between central corneal thickness, rim area, mean RNFL thickness, RNFL cross sectional area with all groups was described and a negative correlation between maximum cup depth and mean cup depth reported in an analysis of all groups combined (Gunvant et al., 2008). The latter results suggest that as corneal thickness decreases, the lamina is probably more compliant, supporting Lesk's finding using slightly different methodology (Lesk et al., 2006). Very recently Mokbel et al. described an association between thin CCT measured by pachymetry and with vertical and horizontal cup disc ratio, smaller optic disc surface area assessed by HRT II and increased mean deviation on visual field analysis in an analysis of 80 eyes of 50 POAG patients in Oman (Mokbel and Ghanem, 2010). The Bridlington Eye Assessment Project, which was established to screen for ocular disease in white Caucasian individuals over 65 years of age in North Humberside, found no significant correlation between any global optic disc parameters assessed by Heidelberg Retinal Tomography and central corneal thickness measured using ultrasonic pachymetry, which supports our findings of no association between central corneal thickness and optic nerve head parameters. In a histomorphometric study of non-glaucomatous human eyes, neither Jonas et al who studied cadaver eyes, nor Ren et al who studied Chinese eyes enucleated due to malignant melanoma, could find an association between a central corneal thickness with lamina cribrosa or peripapillary sclera (Jonas and Holbach, 2005, Ren et al., 2010).

We went further than other publications, and in addition to global optic disc parameters, investigated the relationship between peripapillary atrophy and central corneal thickness.

A number of studies have suggested an association between PPA and glaucomatous optic neuropathy (Primrose, 1970, Wilensky and Kolker, 1976, Nevarez et al., 1988, Buus and Anderson, 1989, Jonas et al., 1992, Jonas and Xu, 1993, Jonas et al., 1989, Jonas and Naumann, 1989, Araie et al., 1994, Lee et al., 2002, Xu et al., 2007c). The size of both alpha and beta PPA has been found to be greater in both size and frequency in glaucomatous eyes compared to the normal population (Jonas et al., 1989, Jonas and Naumann, 1989). Beta zone PPA has been correlated with the position of visual field defects (Park et al., 1996). The extent and area of PPA has been found to be greater in eyes with optic disc hemorrhages (Ahn et al., 2004, Sugiyama et al., 1997) associated with the progression of ocular hypertension to glaucoma (Tezel et al., 1997b, Tezel et al., 1997c), as well as the advancement of glaucomatous optic neuropathy and visual field deterioration (Hayreh et al., 1998, Uchida et al., 1998), (Teng et al., 2010). Models of optic nerve head biomechanics have demonstrated that the mechanical stress induced by a given IOP on the optic nerve head is greater in relatively thin peripapillary sclera (Burgoyne et al., 2005). PPA is hence an important factor which influences optic nerve head biomechanics. However, very few studies have explored the relationship between CCT and PPA. Tomais et al. (Tomais et al., 2008) investigated the relationship between CCT, optic disc area, optic cup area, and peripapillary atrophy in three groups of 30 individuals (patients with glaucoma, ocular hypertension and disease free individuals) in Athens but found no correlation between central corneal thickness and peripapillary atrophy, though they did find a negative correlation between PPA and disc area in eyes with ocular hypertension. The difference between PPA area between the three groups was not statistically significant. We too found no relationship between the area or extent

of PPA to central corneal thickness, which is against our expectations of a thinner cornea being associated with an optic nerve head which may be more vulnerable to IOP induced stress. Teng et al, (Teng et al., 2010) found a very weak agreement between the presence of a thin cornea (<525µm) and the presence of beta zone PPA in a study involving 245 patients with open angle glaucoma. To our knowledge there have only been two other population based studies investigating at peripapillary atrophy in a normal population but neither investigated the relationship between PPA and central corneal thickness (Ramrattan et al., 1999, Xu et al., 2007c).

Because of the difficulties of measuring the lamina cribrosa and peripapillary sclera in vivo, and as the corneoscleral envelope is a continuous structure, some studies have attempted to find more indirect evidence for an association between CCT and optic nerve head biomechanics by examining scleral thickness or, as myopia is an independent risk factor for glaucoma, and as the lamina cribrosa between myopic eyes is thinner than non-myopic eyes, between CCT and refractive error/axial length (Jonas et al., 2004). These studies have produced varied results with some supporting a relationship between CCT and scleral thickness (Albekioni et al., 2003), (Oliveira et al., 2004, Oliveira et al., 2006) but not others (Cho and Lam, 1999, Oliveira et al., 2004, Shimmyo and Orloff, 2005, Oliveira et al., 2006). Population based studies investigating the relationship between central corneal thickness and these quantitative traits are also now beginning to emerge. Meiktila Eye Study from Myanmar (Casson et al., 2008), and the Singapore Malay Eye Study (Su et al., 2009) report a positive association between CCT and spherical equivalent refraction and CCT and axial length respectively. The Tajimi Eye

Study (Suzuki et al., 2005) found a positive relationship between refractive error and CCT but only in men. In contrast, the Beijing Eye Study did not find an association between CCT and refractive error (Zhang et al., 2008) which is in keeping with our findings in the Orcadian population of no statistically significant relationship between central corneal thickness and refractive error or axial length.

Finally, we found no relationship between CCT, corneal curvature or white-on-white. Again, some studies have supported a relationship between these traits whilst others have found no such association. The Tajimi Eye Study (Suzuki et al., 2005) which investigated a population of Japanese ancestry, Shimmyo et al. in a study of individuals of self reported Caucasian, Asian, Hispanic and African American ancestry (Shimmyo et al., 2003), Tong et al in a group of Singaporean school children all reported an association between corneal thickness and curvature. The Reykjavik Eye Study (Eysteinsson et al., 2002) as well as a number of smaller studies found no such association (Chen et al., 2009).

TABLE 6.6: CORNEAL DIAMETER IN SELECTED POPULATIONS

STUDY	YEAR	POPULATION/ ETHNIC ORIGIN	AGE RANGE /MEAN AGE	METHOD	MEAN HORIZONTAL CORNEAL DIAMETER ±SD
(Alsbirk)	1975	Inuit	15+	Wessley Keratometer	10.98±0.42
(Baumeister et al., 2004)	2004	White Caucasian Frankfurt, Germany	20-79	Calliper (Holladay-Godwinguage) Orbscan II IOL master	11.91±0.71 11.80±0.60 12.02±0.38
(Rufer et al., 2005)	2005	White Caucasian Hanover, Germany	10-80	Scanning-slit topographer (Orbscan II)	11.71±0.42
(Tananuvat and Pansatiankul, 2005)	2005	Thailand	20-60	Orbscan II	11.61±0.36
(Kohnen et al., 2006)	2006	White Caucasian Frankfurt Germany	23-55	IOL master Orbscan II	12.17 ±0.45 11.84 ± 0.41
(Pinero et al., 2008)	2008	White Caucasian Alicante, Spain	20-51	Digital calliper from CSO corneal topography system	12.25±0.49
(Salouti et al., 2009)	2009	Iranian Same cohort for all three methods	27.4±7.2	Dual-Scheimpflug System Scanning-slit topographer (Orbscan II) EyeSys Corneal Analysis System	12.01±0.61 11.67±0.29 12.09±0.87
(Hashemi et al., 2009c)	2009	Iranian	14-81	Orbscan II	11.68±0.46

Even though an understanding of “normal” corneal width is invaluable in ophthalmology, it has been poorly investigated. Horizontal corneal width is important for a number of reasons. Various cut offs of corneal width are used for the definition/diagnosis of ocular conditions such as micro and megalocornea (Meire, 1994); it is used to diagnose and subsequently monitor congenital glaucoma (Meire, 1994); Corneal diameter measurements are used in to improve the accuracy of IOL power calculations in third generation formulas (Holladay et al., 1996), for estimating the size of the size of ciliary sulcus and anterior chamber width before the implantation of sulcus-fixated lenses (Price and Parker, 1997). Corneal diameter is also important in the preoperative evaluation of patients who wish to undergo refractive surgery (Darlington and Davis, 2008). Corneal diameter is also important in glaucoma - the diameter of the cornea in relation to glaucoma has been described as far back as the 1800s when Priestly Smith reported a smaller corneal diameter in eyes with primary glaucoma compared to normal eyes (Alsbirk, 1975). A number of studies have investigated corneal diameter and perhaps their methods though novel at the time, compared to current technology, could be considered to be of limited precision (Rufer et al., 2005). Friede for example, reported a mean corneal diameter of 11.58 mm after measuring the horizontal and vertical corneal diameters of nearly 11,000 individuals in 1933 using Wessely’s keratometer, a device which allowed the measurement of corneal diameter by looking through a tube with a millimeter rule at one end and a lens at another (Alsbirk, 1975). Alsbirk used Wessely’s keratometer to investigate corneal diameter in Greenland in the 1970s, and established that there was a difference between corneal diameter between those of Inuit ancestry compared to Danish ancestry (Alsbirk, 1975). The first

population based study investigating corneal width in a normal population using current technology was Rufer et al (Rufer et al., 2005). Using the Orbscan II, a non contact corneal topography system using a mixture of reflective (placido) and scanning slit technology (Rufer et al., 2005, Swartz et al., 2007), investigated the horizontal corneal diameters of 231 male and 140 female white Caucasian volunteers in Hanover, Germany. Volunteers were aged 10 to 80 years. Average corneal diameters 11.71±0.42 mm, giving a range of 10.87 to 12.55. The mean corneal diameter found in our study population, 12.3mm for the right eye, and 12.2mm in the left eye, was higher than the majority of published studies, and certainly higher than all studies that are population based. If a reference range was calculated using our data, the range defined as mean±2 standard deviations, the upper limit of normal would be 13.2mm in the right eye and 13 mm in the left.

Though accurate data about the possible normal dimensions of the anterior segment, and their associations with other ocular biometric and demographic variables would be useful, especially in an era of increasing refractive surgery, the number of studies investigating the relationship between corneal thickness and corneal diameter in adults using current technology are limited (see table 6.6). Three population based studies and a number of other smaller studies, have published normative data on corneal diameter in adults but few of these studies have explored the relationship between corneal diameter, demographic variables or other ocular parameters (Alsbirk, 1975, Baumeister et al., 2004, Rufer et al., 2005, Tananuvat and Pansatiankul, 2005, Kohnen et al., 2006, Pinero et al., 2008, Hashemi et al., 2009b, Salouti et al., 2009). We investigated the influence of

a number of demographic and ocular biometric variables on corneal diameter and found only corneal curvature and anterior chamber depth had a statistically significant correlation with corneal diameter. Neither gender nor age in adults correlated significantly with diameter. Rufer et al. in contrast found corneal diameter decreased slightly with age but there was no difference between genders (Rufer et al., 2005). In a Tehran-based study, Hashemi et al. found corneal diameter differed significantly between genders but was not correlated to age (Hashemi et al., 2009b). A small number of studies, in keeping with our findings, have reported a positive correlation between corneal diameter and anterior chamber depth (Hosny et al., 2000, Kohnen et al., 2006, Hashemi et al., 2009b). Hosny et al (Hosny et al., 2000) investigated the relationship between several ocular biometric parameters in 211 individuals in Alicante and found no relationship between corneal diameter and CCT or axial length. Corneal diameter did however, correlate with anterior chamber depth.

Limitations of our study in the context of the corneal parameters of thickness and width, we share with other published studies in this field. There is evidence to suggest that corneal thickness, like IOP, shows both diurnal and seasonal variation, so any solitary or group of measurements taken at a point in time only represents “true” corneal thickness to a certain extent (Doughty and Zaman, 2000). The problems with study comparison are similar to those faced in comparing studies on intraocular pressure. The current clinical gold standard for measuring corneal thickness is ultrasonography - the method used in our study. Other methods used in the literature, include, scanning slit topography, specular microcopy and other forms of optical pachymetry. These methods are of

variable comparability. Scheimpflug anterior segment photography, more commonly used for the assessment of lens thickness, is a less common method of assessing corneal thickness (Friedman and He, 2008, Wegener and Laser-Junga, 2009). It is a form of corneal tomography using a geometrical principle that was first described by an Austrian Naval Officer Theodor Schiefpflug in the late 1800s. This rule describes the orientation of the focus plane of an optical system if the image and lens planes are not parallel to each other. In this situation, a tangent can be drawn from the image, object and lens planes which allows the calculation of the point of "best focus". The EAS-1000 Anterior Eye Segment Analysis System (Nidek Co Ltd, Japan) used in the Reykjavik Eye Study, is based on this principle (Eysteinsson et al., 2002). It acquires a single image from which CCT can be calculated (Barkana et al., 2005). More recent anterior segment imaging systems, such as Pentacam, acquires multiple images of the anterior segment. Specular microscopy works on the principle of specular reflection – the phenomenon when objects are observed using light reflected off the different interfaces of an object formed from material of different refractive indices (such as the cornea) (Cavanagh et al., 2000, Modis et al., 2001a, Swartz et al., 2007). The Topcon SP-2000P, a non contact specular microscope (Topcon, Tokyo, Japan), used in the Tajimi Eye Study, (Suzuki et al., 2005) is also capable of pachymetry by calculating focal distances (from epithelium to endothelium) which can be used to calculate corneal thickness (Modis et al., 2001a). Foster et al (Foster et al., 1998) used another form of optical pachymeter the Depth Measuring Device I; Haag-Streit, Bern, Switzerland, which is also dependent on specular reflections of corneal interfaces, but instead uses a measuring device mounted on a slit lamp (Bourne and Alsbirk, 2006). These techniques

show variable agreement, and the evidence suggests, should not be used interchangeably (Modis et al., 2001a, Modis et al., 2001b). For example, in a study by Prospero Ponce and colleagues, ultrasound pachymetry was found to produce readings that are higher than Scheimpflug measurements of central corneal thickness (Prospero Ponce et al., 2009). In another study, central corneal thickness was found to be 12-24μm greater when measured using scanning slit lamp corneal topography than with non-contact specular microscopy (Sanchis-Gimeno et al., 2006).

The similar problems of study comparison can be found in studies investigating corneal diameter – differing methods of procurement, measurement, populations etc. Baumeister et al compared 2 automated (Orbscan, IOLmaster) and two manual (surgical callipers and Holladay-Godwin Corneal Gauge) methods of measuring corneal diameter and found manual methods of measuring corneal diameter were comparatively less precise, with the Carl Zeiss IOLmaster, the instrument used in our study, being the most reliable (Cavanagh et al., 2000).

Comparison between studies is difficult due to differences in methodology but overall our findings demonstrate that though values obtained for corneal parameters in the Orcadian population may differ from other published reports, they are not dissimilar. There is a statistically significant association between central corneal thickness, which is highly relevant clinically but we could not find association between central corneal thickness and other variables. In particular we could not find a relationship between central corneal thickness, optic nerve head parameters, refractive error or axial length.

Corneal diameter was related to corneal curvature and anterior chamber depth but not to any of the other variables we investigated.

CHAPTER 7

- RESULTS -

INTRAOCULAR PRESSURE IN THE SCOTTISH POPULATION ISOLATE OF ORKNEY

7.1 RESULTS

The intraocular pressure of the eye is generated and modulated by the formation of aqueous humour in the ciliary body, which flows from the posterior to the anterior chamber of the eye, and exits via the trabecular meshwork and uveoscleral pathways (Hart, 1992). Figure 7.1 shows the distribution of IOP in our sample, for both right and left eyes with normal distribution curves superimposed.

Mean IOP for right eyes was 14.8mmHg (95% confidence intervals 14.4mmHg, 15.2mmHg), and for left eyes 15.1mmHg (95% confidence intervals 14.7mmHg, 15.6mmHg). This slight difference in mean IOP between left and right eyes was statistically significant at the 5% level (paired t-test, p-value = 0.021) but not clinically significant (mean difference = 0.311 mmHg, 95% confidence interval for mean difference -0.576mmHg, -0.046mmHg). Mean IOP in men in the right eye was 14.7mmHg, in the left 15.0mmHg. In women mean IOP was 15mmHg in the right eye

and 15.3mmHg in the left eye. The difference in IOP between men and women was not significant (p = 0.643).

Bivariate analysis only found a statistically significant association between IOP and CCT (p=0.004) and IOP and age (p=0.005), but not with any of the other ocular biometric parameters including axial length or any optic nerve head parameter listed in table 7.2. On multivariate analysis, age and central corneal thickness were found to be significantly associated with IOP at the 5% level (p = 0.032 and p=0.004 respectively). Table 7.2 summarizes the results of multivariate analysis of IOP as the dependent variable in right eyes.

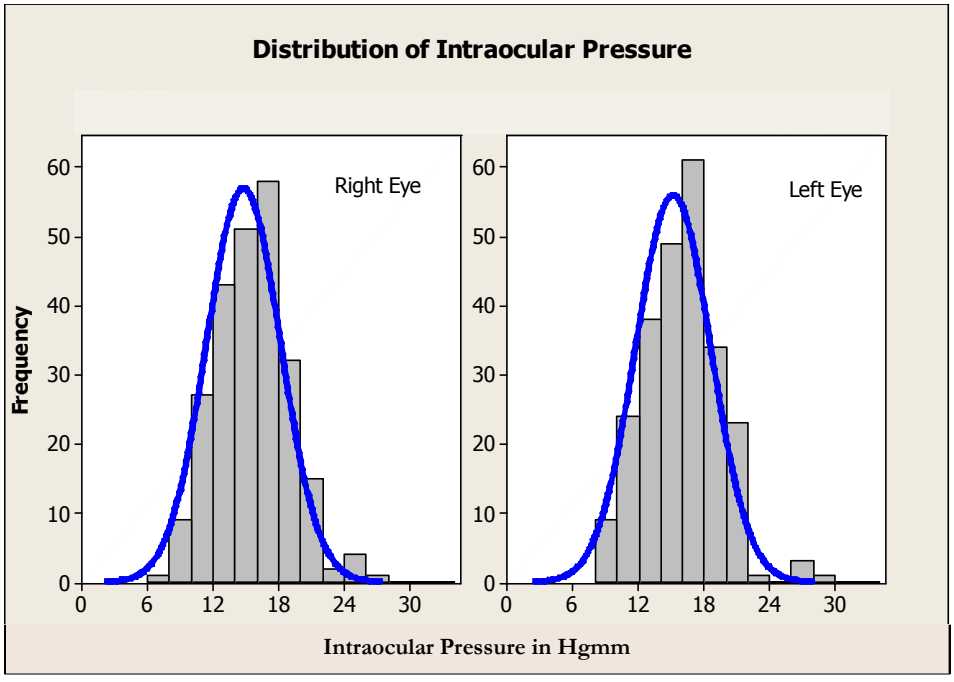

FIGURE 7.1: DISTRIBUTION OF INTRAOCULAR PRESSURE IN THE STUDY POPULATION

TABLE 7.1: SUMMARY STATISTICS FOR INTRAOCULAR PRESSURE

	Right Eye (mmHg)	**Left Eye** (mmHg)
Mean	14.8	15.1
95% Confidence interval for mean	14.4 -15.2	14.7 - 15.6
Standard Deviation	3.4	3.5
Range	7 - 28	8 - 29
Males Mean (95% CI)	14.7 (14.0-15.4)	15.0 (14.3-15.7)
Females Mean (95% CI)	15.0 (14.5-15.5)	15.3 (14.8-15.8)

TABLE 7.2: MULTIVARIABLE ANALYSIS FOR INTRAOCULAR PRESSURE

VARIABLE	**COEFFICIENT**	**STANDARD ERROR**	**P-VALUE**
Age	0.0571	0.02644	0.032
Gender	0.2585	0.5564	0.643
Spherical Equivalent	0.032	0.087	0.714
Central Corneal Thickness	1.2978	0.4479	0.004
Keratometry	228.0	119.6	0.059
Corneal Diameter	1.2113	0.8545	0.159
Anterior Chamber Depth	0.0162	0.0799	0.839
Axial Length	0.874	1.063	0.412
Nerve Fiber Layer Thickness	0.0799	0.0709	0.262
Rim Area	-0.0767	0.0836	0.361
Cup Area	0.0965	0.1553	0.535
Maximum Cup Depth	-10.99	21.37	0.608
PPA Area	-0.1244	0.2243	0.580
PPA peripheral extent	0.0936	0.1946	0.631

7.2 DISCUSSION

The role of ocular tension, or IOP in the pathogenesis of glaucoma, has been the focus of research for many centuries (Lascaratos and Marketos, 1988b, Fronimopoulos and Lascaratos, 1991, Nathan, 2000, Cohen, 2001, Drews, 2006, Tsatsos and Broadway, 2007), (Kronfeld and 2009). Over the last few decades a number of population based studies have investigated the distribution of IOP in the normal population. Table 7.3 below summarizes some of these main studies.

TABLE 7.3: INTRAOCULAR PRESSURE IN SELECTED POPULATIONS

STUDY	YEAR	POPULATION	AGE RANGE /MEAN AGE	METHOD OF MEASUREMENT	MEAN IOP ±SD
Joensusu, Finland. (Katavisto and Sammalkivi, 1964)	1964	Finnish	40+	Schiotz	16.0±2.72
De Moine, Iowa (Armaly, 1965)	1965	White Caucasian	20-80	GAT	Male: 15.6±3.22 Female: 16.1±3.23
Rhonda Valley, Wales (Hollows and Graham, 1966)	1966	Welsh	40-74	GAT and Shiotz	15.9±2.87
Bedford Glaucoma Survey (Bankes et al., 1968)	1968	White Caucasian	20+	GAT	16.3±3.43
Jamaica (Wallace and Lovell, 1969)	1969	African-Caribbean	35-74	GAT	Male: 16.8± 2.8 Female: 16.5 ± 2.9
Umanak, Greenland (Alsbirk, 1970)	1970	Inuit-Caucasian	20+	Shiotz	15.7±3.0
Dalby Eye Study (Bengtsson, 1972)	1972	White Caucasian	8-99	GAT/Schiotz	15.0±5.0
Framingham Eye Study, USA. (Kahn et al., 1977a)	1977	Majority White Caucasian	52-85	GAT. Shiotz if GAT not possible	17.0±4.1
Nagoya, Japan. (Shiose and Kawase, 1986)	1986	Japanese	-	Shiotz/non contact tonometry	Male: 14.6±2.5 Female: 15.0±2.3
Baltimore Eye Survey (Sommer et al., 1991)	1991	46% White Caucasian 54% African Caribbean	≥40	GAT	White Caucasian 17.2±3.3 African Caribbean 16.0±4.2

Beaver Dam (Klein et al., 1992a)	1992	White Caucasian	43-84	GAT	Male: 15.3±3.4 Female: 15.5±3.3
Rotterdam Eye Study Netherlands (Dielemans et al., 1994)	1994	White Caucasian	≥55	GAT	14.(NR)
Blue Mountains Eye Study (Mitchell et al., 1996)	1996	White Caucasian	49-79	GAT	16.1±2.9
Barbados Eye Study (Leske et al., 1997)	1997	93% African Caribbean 4% Mixed African Caribbean +White Caucasian 3% White Caucasian or other	40-80	GAT	African Caribbean 18.7±5.2 Mixed: 18.2± 3.8 White Caucasian or other: 16.5±3.0
Vellore, India (Jacob et al., 1998)	1998	Indian	30-60	GAT	15.48±3.6
Egna-Neumarkt study (Bonomi et al., 1998)	1998	Italian	>40 years	GAT	Male: 15.14± 2.8 Female: 14.94±2.6
Visual Impairment Project, Australia (Weih et al., 2001)	2001	Classified by place of birth: 68% Australia/New Zealand 13.4% Greece/Italy 8.7%British Isles 9.9% Elsewhere	≥40	GAT	14.3 ±1.5
KwaZulu-Natal, South Africa (Rotchford and Johnson, 2002)	2002	South African (Zulu)	>40	Tono-Pen XL	13.9±3.4
Tehran Eye Study (Hashemi et al., 2005)	2005	Persian (Indo-European)	≥40	GAT	15.1±2.9
Beijing Eye Study, China (Xu et al., 2005)	2005	Chinese	40-101	Non contact pneumatonometry	16.1±3.4
Ethiopia (Gelaw et al.)	2010	African	42.6±16.7	GAT	13.4±2.8

Since the late 1950's, these population based studies, have demonstrated that mean IOP, it's distribution and it's relationship to other variables such as age, and gender, can vary between populations. For example, in the Barbados Eye Study (Leske et al., 1997), a population based study which was established to investigate the prevalence of open

angle glaucoma and other ocular conditions in the Antilles, found that IOP was significantly higher in individuals of self reported African-Caribbean origin (mean IOP±SD: 18.7±5.2mmHg), compared to those of White Caucasian ancestry (mean IOP±SD: 16.5±3.0mmHg). Similar findings were reported by the Health and Nutrition Survey (Klein and Klein, 1981, Hiller et al., 1982) but the Baltimore Eye Study however, found that IOP was similar in volunteers of African-Caribbean and White Caucasian ancestry (Sommer et al., 1991).

In the majority of studied populations, intraocular pressure amongst normal individuals also shows a right skewed distribution. Various theories have arisen to explain this phenomenon. In the late 1950s, Wolfgang Leydhecker and associates suggested that this skewness could be explained by the presence of two sub-populations, the amalgamation of which would form the final right skewed curve (Davanger and Holter, 1965). The primary "collective" consists of a population with IOPs that follow a normal distribution curve centered around a mean of 16mmHg. The secondary population consists of a group with higher IOPs, ranging from 24 to 41mmHg. The distribution of these pressures are not symmetric about the mean but skewed towards higher pressures. This secondary collective is believed to be formed by glaucomatous or potentially glaucomatous eyes. However, we now know that the relationship between IOP and glaucoma is not that straight forward, and the statistically designated cut-offs between normal and pathological are too simplistic to distinguish between healthy eyes and ones with disease potential. Furthermore, IOP, like blood pressure, is a physiological property, governed by the interaction of different factors. With this idea in mind, Martin

Davanger and Oivin Holter showed that the skewed distribution for IOP demonstrated by various population based studies could also be explained by fluid dynamics, and related the skewed nature of IOP distribution to physical ocular properties such as effective pore diameter (Davanger and Holter, 1965). Davanger and Holter demonstrated that if the diameter of outflow pathways within the trabecular meshwork ("effective pore diameter"), were presumed to have a normal distribution within the population, for both physiological and more complex mathematical reasons, IOP would be expected to show a skewed distribution. This explanation supports our increasing understanding of IOP as a continuum, abrogating the need for Leydecker's assumption of two separate populations, one with "normal" IOP mechanisms and another with abnormal IOP mechanisms.

These findings have not been repeated in all populations. In South East Asian populations, such of those of the Tajimi (Fukuoka et al., 2008) and Nagoya (Shiose, 1984) the distribution of IOP follows a different course – left skewed with IOP decreasing with age rather than increasing. Other South East Asian populations have reported inverse "U" shaped distributions of IOP (Xu et al., 2005, Wong et al., 2009), Wong, 2009). The Beijing Eye Study (Xu et al., 2005, Xu et al., 2009), for example, a population based cohort study, measured IOP using non-contact pneumotonometry in a group of 4439 individuals from Northern China, IOP showed a reversed-U shaped course, with an increase in IOP from the subgroup 40 to 44 years to 60 to 64 years, followed by a decrease in IOP from that subgroup to the sub-group ≥75 years.

The distribution of IOP of both eyes in the Orkney population follows those of other White Caucasian populations. It is unimodal, and skewed to the right, with a mean and standard deviation, and range comparative to other White Caucasian populations. IOP in the Orkney sample also shows a statistically significant correlation with age which is in keeping with other populations such as those of White Caucasian and African-Caribbean ancestry (Armaly, 1965, Klein et al., 1992a, Leske et al., 1997, Bonomi et al., 1998) but not of South East Asian ancestry. (Shiose, 1984, Fukuoka et al., 2008), (Xu et al., 2005, Wong et al., 2009) In some studies of White Caucasian populations, this association is not significant (Kahn et al., 1977a). Aging is associated with a number of changes in both ocular and non ocular tissue. Age related changes have been described in the trabecular meshwork which increase the resistance of the meshwork to aqueous outflow (Tamm and Fuchshofer, 2007). These changes may explain part of the age related increase in IOP observed in these studies. Changes in the cornea such as increased cross linking between collagen fibrils have also been observed with age and these changes may contribute to some of the biomechanical changes of the cornea associated with age (Kotecha, 2007). In addition to corneal thickness, other biomechanical parameters may also influence the measurement of IOP. The increased corneal rigidity associated with age may hence induce further measurement errors and contribute to this association (Kotecha et al., 2005).

We did not find a significant relationship between gender and IOP though there is evidence that oestrogen receptors can be found in the ciliary body and outflow tract and it is possible these could influence glaucoma via aqueous formation (Ogueta et al., 1999,

Lee et al., 2003). There is also some evidence that female sex hormones influence intraocular pressure (Altintas et al., 2004, Sator et al., 1997). Our findings are supported by some population based studies (Klein et al., 1992a, Giuffre et al., 1995) but not others (Armaly, 1965, Hollows and Graham, 1966, Leske et al., 1997, Wallace and Lovell, 1969).

The only ocular factor found to be associated with IOP in our study was CCT. This finding has been reported in many others, in both population based as well as smaller studies (Wolfs et al., 1997, Dohadwala et al., 1998, Foster et al., 1998, Bhan et al., 2002, Eysteinsson et al., 2002, Ko et al., 2005, Li et al., 2002, Lleo et al., 2003, Foster et al., 2003), (Suzuki et al., 2005, Doughty and Jonuscheit, 2007, Zhang et al., 2008, Su et al., 2009, Gelaw et al.), though not all populations have reported this association (Nemesure et al., 2003). Perusing the IOP-CCT relationship has been a means of assessing the effect of CCT on IOP (Doughty and Zaman, 2000, Bhan et al., 2002, Doughty et al., 2002) (Doughty and Jonuscheit, 2007). This relationship we discussed at some length in Chapter 1, then revisited in chapter 6. In brief, the measurement of intraocular pressure by some forms of tonometry, especially Goldman applanation tonometry is influenced by the corneal thickness (Bhan et al., 2002, Kniestedt et al., 2008). In Goldman Applanation Tonometry, a force is used to applanate a known area and then used to calculate pressure as pressure is equivalent to force divided by area. The “known area” was calculated by Goldman and Schmidt as one where the effects of surface tension created by the tear meniscus would abrogate or reduce the effects of the rigidity of the cornea. Unfortunately in this calculation, Goldman and Schmidt assumed a central

corneal thickness of 500µm and further assumed that there would be little inter or intra-population variability in this parameter. (Hart, 1992, Whitacre and Stein, 1993, Chihara, 2008). A number of studies where manometry and tonometry have been performed concurrently have demonstrated that central corneal thickness has an impact on tonometry, and overall, thick corneas overestimate IOP and thinner than "average" corneas underestimate IOP (Ehlers et al., 1975, Whitacre et al., 1993, Doughty and Zaman, 2000), (Feltgen et al., 2001, Kohlhaas et al., 2006). This relationship does not hold true for all populations. For example, in the Barbados Eye Study, the relationship between IOP and CCT in volunteers of African Caribbean or "mixed" ancestry was not statistically significant, though the relationship was statistically significant for those of White Caucasian ancestry (Nemesure et al., 2003).

A number of studies have identified myopia as a risk factor for open angle glaucoma (Daubs and Crick, 1981, Ponte et al., 1994, Mitchell et al., 1999, Grodum et al., 2001, Yoshida et al., 2001, Ramakrishnan et al., 2003, Wong et al., 2003, Xu et al., 2007b). Furthermore, ocular tension has been postulated as a possible mechanism for myopia and axial elongation (Pruett, 1988, Nomura et al., 2004, McMonnies, 2008). A number of studies, most not taking CCT into account, have shown a correlation between intraocular pressure and myopia (Abdalla and Hamdi, 1970, David et al., 1985) (Quinn et al., 1995, Mitchell et al., 1999, Wong et al., 2003, Nomura et al., 2004) However, Edwards and Brown (Edwards and Brown, 1996) found that myopia preceded a higher IOP rather proceeded it, suggesting that IOP may not be a cause of axial elongation but a result of it. This finding has not been universal (Goss and Caffey, 1999). In theory, if a

cornea is steeper, a greater force needs to be applied in order to obtain the required applanation area. Some studies have supported this theory (Liu and Roberts, 2005, Harada et al., 2008) whereas others have not found such a relationship (Francis et al., 2007, Saleh et al., 2006).We did not find any relationship between IOP and refractive error, axial length or corneal curvature which is keeping with a number of other published studies (Lee et al., 2004b, Manny et al., 2008, Francis et al., 2007, Saleh et al., 2006). The reasons for these possible differences are discussed below and in chapter 9.

Since the 10th century, and the first documented putative relationship between glaucoma and intraocular pressure was described by the Arabian surgeon Al-Tabari (Cohen, 2001), raised intraocular pressure has been intimately associated with the pathogenesis of glaucoma (Halpern and Grosskreutz, 2002, Flammer and Mozaffarieh, 2007). Population based studies have demonstrated that increased IOP is related to an increased prevalence of open angle glaucoma in a dose dependent manner (Sommer et al., 1991, Klein et al., 1992a, Klein et al., 1992b, Mitchell et al., 1996, Foster et al., 2003, Iwase et al., 2004, Vijaya et al., 2005). A number of large multi-centered randomized controlled trials have demonstrated that the onset/progression of glaucomatous optic neuropathy can be reduced by lowering IOP (2000, Feiner and Piltz-Seymour, 2003, Heijl et al., 2002, Burr et al., 2005, Maier et al., 2005, Vass et al., 2007). A number of animal models for the disease have corroborated and provided further means of investigating the pathogenesis of glaucoma in vivo. (Lindsey and Weinreb, 2005, Morrison, 2005, Rasmussen and Kaufman, 2005, Weinreb and Lindsey, 2005). Overall, raised IOP induces a series of events which culminates in the death of retinal ganglion cells by

apoptosis. (Guo et al., 2005). The association between raised IOP and optic nerve head parameters in eyes with open angle glaucoma is well established (Sommer et al., 1991, Leske et al., 1997, Halpern and Grosskreutz, 2002, Flammer and Mozaffarieh, 2007). Even in glaucoma free eyes, a number of population based studies have found correlations between IOP and certain optic nerve parameters (Varma et al., 1995, Leske et al., 1997, Leibowitz et al., 1980, Abe et al., 2009). For example, in the Baltimore Eye Study, higher IOP was correlated with a smaller neural retinal rim area (Varma et al., 1995). In the Tajimi Eye Study, IOP was found to be positively correlated to cup area and cup to disc area ratio and negatively correlated to rim area, rim volume and retinal nerve fiber layer thickness (Abe et al., 2009). This has not been the case in all population based studies however (Klein et al., 1989, Varma et al., 1995, Ruangvaravate and Neungton, 2008). We too found no relationship between IOP and optic nerve head parameters including peripapillary atrophy in our population, supporting the latter rather than the former reports. There are many possible reasons for these differences. These are discussed below and in chapter 9.

One of the greatest problems in this area of research is that these studies are difficult to compare. Comparison is hampered by the same problems that hinder the comparison of other studies looking at ocular QTs– such as lack of homogeneity of method and that many are not population based. The clinical gold standard for measuring IOP is Goldman Applanation tonometry (GAT), which we used in our study. Older population based studies looking at population distributions of IOP have used Schiotz tonometry or a mixture of Schiotz and GAT (table 7.3). More recent studies, have used

pneumotonometry or the tonopen. Schiotz tonometry is a form of indentation tonometry (as opposed to applanation tonometry used by Goldman) (Kniestedt et al., 2008). The basic principle that governs indentation tonometry is quite simple – if the internal pressure of a fluid or gas filled object is low, then a given force will cause greater indentation than if this pressure was high. The Schiotz tonometer, first developed in 1905, was gradually replaced the Malakoff tonometer. The Schiotz tonometer is used with the volunteer or patient supine. Gravity provides the "known force" for a weighted metal plunger attached to a scale. The lower the IOP, the deeper the plunger descends into the anterior segment. For the pointer on the scale to move 1 unit, the plunger must descend 0.05mm. Despite these simple principles, its use is complex. The scale readings are related in a logarithmic rather than in simple a linear manner to IOP; Nomograms, developed using cadaver eyes (Friedenwald, 1957, McBain, 1957), must be used to convert scale readings into pressure readings; Additional weights must be added to the plunger to read higher pressures (above the scale reading 3, which gives a pressure of 24.4 with 5.5g using the Friedenwald nomogram). Shiotz also assumed that ocular rigidity was a constant when designing his tonometer. Scleral rigidity is not the same in all eyes. Myopes for example have lower scleral rigidity than hyperopes and emmetropes – IOP hence tends to be underestimated (Mc, 1958). Pressure readings obtained via Shiotz tonometry also show a poor correlation to the current gold standard, Goldman Applanation Tonometry (Krieglstein and Waller, 1975).

The gold standard for measuring IOP in the laboratory is manometry (Kniestedt et al., 2008). Manometric studies have demonstrated that GAT is more accurate than the Tono-

pen in measuring IOP (Eisenberg et al., 1998). Studies have shown that pneumotonometry tends to overestimate IOP at lower pressures and under estimate IOP at higher pressures. IOP values obtained via pneumotonometry were also consistently higher than that by GAT (Sanchez-Tocino et al., 2005). Clinical studies have shown that the reliability of the tonopen is also less than that of GAT (Boothe et al., 1988), with variable results for pneumotonometry (Zadok et al., 1999, Tonnu et al., 2005, Regine et al., 2006). The accuracy of even the gold standard GAT is influenced by a variety of factors (Whitacre and Stein, 1993). GAT overestimates IOP in non-oedmatous thick corneas and under estimates IOP in oedemetaous and thinner corneas (Ehlers et al., 1975). Nomograms are available for correcting IOP according to CCT but their acceptance and use is far from universal (Chihara, 2008, Doughty et al., 2002, Gunvant et al., 2005). None of the population based studies on IOP have taken CCT of their volunteers into account. For this reason, to allow comparison between studies, and also because there is still no method of accounting for CCT which is universally accepted, in our study we have not accounted for CCT in our IOP measurements.

In addition to the problems associated with tonometry, short and long term IOP fluctuations are now well described (Shiose, 1990). There is evidence to suggest that IOP shows not only diurnal variation but seasonal variation as well. It is impractical in a large cohort to limit measurements of IOP to the same time each day or the same period each year. Even if this was done, inter-individual variation would probably be such that we would be unsure at exactly which time of the day that each individual's IOP "peak" and "trough" occurred. 24 hour IOP measurements could be considered the gold

standard, but in a large cohort, this is simply impractical. Hence, any mean IOP measurement for a population, even using GAT, will be an estimate at best.

These findings highlight that intraocular pressure in our sample of the Orcadian population, is not dissimilar to other published White Caucasian populations. IOP in this sample was correlated to both age and central corneal thickness. Clinically, this is a very important finding as these relationships do not hold in all populations (Nemesure et al., 2003, Shiose, 1984, Fukuoka et al., 2008, Xu et al., 2005, Wong et al., 2009). Though no longer considered the sole causative factor, IOP remains an important risk factor for primary open angle glaucoma and remains the only treatable component of the disease. So in the evaluation of subjects for glaucoma and other ocular conditions in the Orcadian population when the assessment of IOP is required, it is important to make the appropriate allowances for age and corneal thickness.

CHAPTER 8

- RESULTS -

OPTIC NERVE PARAMETERS IN THE SCOTTISH POPULATION ISOLATE OF ORKNEY

8.1 RESULTS

The distribution of optic nerve head parameters and nerve fibre layer thickness are shown in the diagrams below and summary statistics in table 8.1. Mean values for these main parameters with their 95% confidence interval in brackets are as follow: optic disc area was 2.10mm² (2.03-2.16mm²) in the right eye, 2,04mm² (1.97-2.11mm²) in the left eye; mean optic rim area was 1.53mm² (1.48-1.58mm²) in the right eye, 1.48mm² (1.43-1.53mm²) in the left eye; mean cup area was 0.57mm² (0.52-0.62mm²) in the right eye, 0.56mm² (0.51-0.61mm²) in the left eye; mean vertical cup to disc ratio in both eyes 0.39 (0.36-0.42); mean nerve fibre layer thickness in both eyes 0.23mm (0.22-0.24mm). The overall prevalence of peripapillary atrophy was 25.0% in right eyes and 25.3% in left eye. 9.6% of effected right eyes, and 9.5% of effected left eyes were unilateral. Mean peripapillary atrophy area in the right eye 0.24mm² (0.14-0.33mm²), left eye 0.28mm² (0.17-0.39mm²). The difference between right and left eyes was only statistically significant at the 5% level for optic disc area in female eyes (p=0.019, paired t-test). For all other parameters, the difference between right and left eyes were not statistically significant at the 5% (p values all > 0.05).

TABLE 8.1: SUMMARY STATISTICS FOR MAIN OPTIC NERVE HEAD PARAMETERS

	Right Eye	Left Eye
Optic Disc Area (mm^2)		
Mean	2.10	2.04
95% Confidence interval for mean	2.03-2.16	1.97-2.11
Standard Deviation	0.52	0.54
Range	0.97	0.64-4.67
Median	2.05	1.99
95% Confidence interval for mode	1.98-2.13	1.88-2.09
Males (Mean , 95% CI)	2.15 (2.05-2.25)	2.09 (1.98-2.19)
Females (Mean , 95% CI)	2.08 (1.99-2.16)	2.02 (1.93-2.11)
Optic Rim Area (mm^2)		
Mean	1.53	1.48
95% Confidence interval for mean	1.48-1.58	1.43-1.53
Standard Deviation	0.39	0.40
Range	0.82-2.87	0.33-2.85
Median	1.46	1.41
95% Confidence interval for median	1.41-1.52	1.37-1.47
Males (Mean , 95% CI)	1.58 (1.50-1.66)	1.53 (1.45-1.62)
Females (Mean , 95% CI)	1.50 (1.44-1.57)	1.45 (1.39-1.52)
Optic Cup Area (mm^2)		
Mean	0.57	0.56
95% Confidence interval for mean	0.52-0.62	0.51-0.61
Standard Deviation	0.41	0.42
Range	0.00-2.87	0.00-2.73
Median	0.51	0.48
95% Confidence interval for mode	0.44-0.55	0.42-0.53
Males (Mean , 95% CI)	0.57 (0.49-0.65)	0.55 (0.47-0.64)
Females (Mean , 95% CI)	0.57 (0.51-0.64)	0.56 (0.49-0.63)

TABLE 8.1 *(CONTINUED)*: SUMMARY STATISTICS FOR MAIN OPTIC NERVE HEAD PARAMETERS

	Right Eye	Left Eye
Vertical Cup to Disc Ratio		
Mean	0.39	0.39
95% Confidence interval for mean	0.36-0.42	00.36-0.42
Standard Deviation	0.23	00.24
Range	0.0-84	0.0-0.9
Median	0.45	0.45
95% Confidence interval for mode	0.43-0.48	0.41-0.48
Males (Mean , 95% CI)	0.40 (0.36-0.44)	0.39 (0.33-0.44)
Females (Mean , 95% CI)	0.39 (0.36-0.43)	0.39 (0.35-0.43)
Nerve Fiber Layer Thickness (mm)		
Mean	0.23	0.23
95% Confidence interval for mean	0.22-0.24	0.22-0.24
Standard Deviation	0.06	0.07
Range	0.01-0.51	0.01-0.45
Mode	0.23	0.24
95% Confidence interval for mode	0.22-0.24	0.23-0.25
Males (Mean , 95% CI)	0.23 (0.22-0.25)	0.24 (0.22-0.25)
Females (Mean , 95% CI)	0.23 (0.22-0.24)	0.24 (0.22-0.25)
Peripapillary Atrophy (mm^2)		
Mean	0.24	0.28
95% Confidence interval for mean	0.14-0.33	0.17-0.39
Standard Deviation	0.73	0.87
Range	0.00-5.05	0.0-7.10
Median	0.00	0.00
95% Confidence interval for median	0.00-00	0.00-0.00
Peripheral Extent (Mean , 95% CI)	42.5 (31.7-53.3)	45.0 (33.9-56.1)
Males (Mean , 95% CI)	0.36 (0.16-0.55)	0.43 (0.19-0.67)
Females (Mean , 95% CI)	0.17 (0.08-0.26)	0.19 (0.09-0.29)

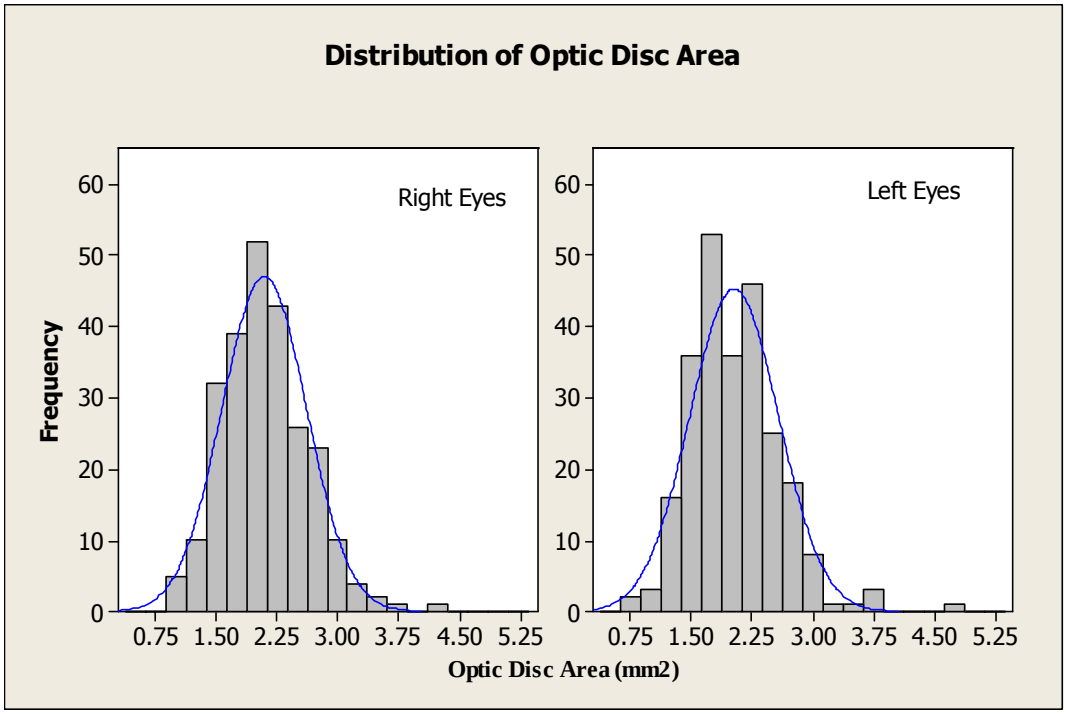

FIGURE 8.1: DISTRIBUTION OF OPTIC DISC AREA

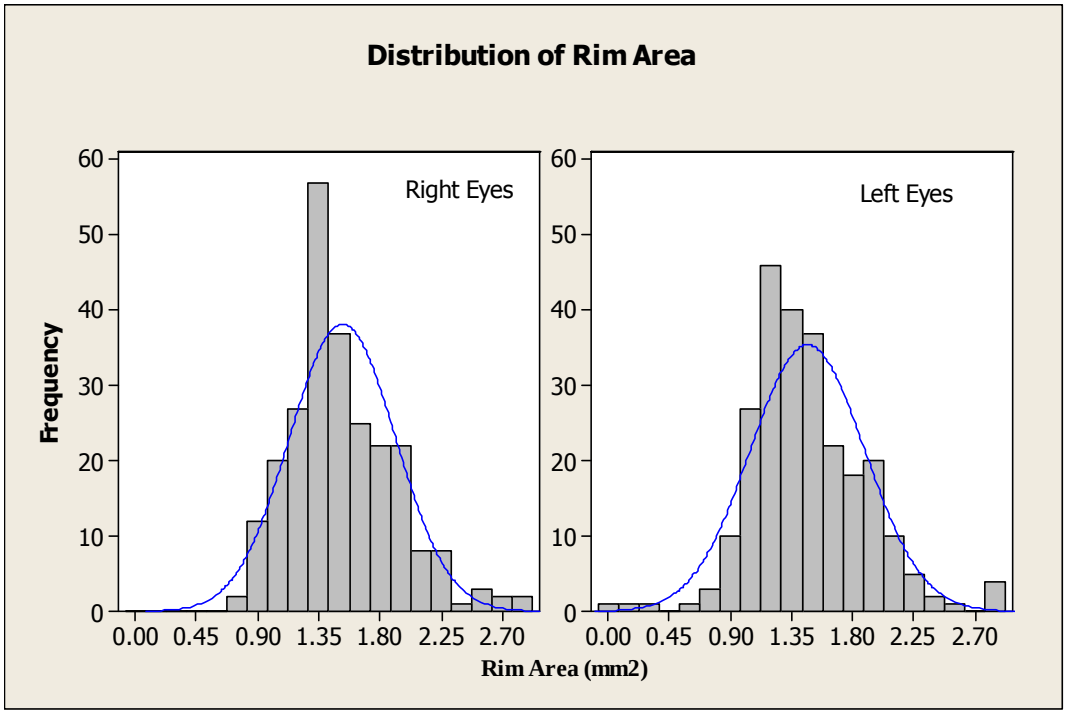

FIGURE 8.2: DISTRIBUTION OF OPTIC RIM AREA

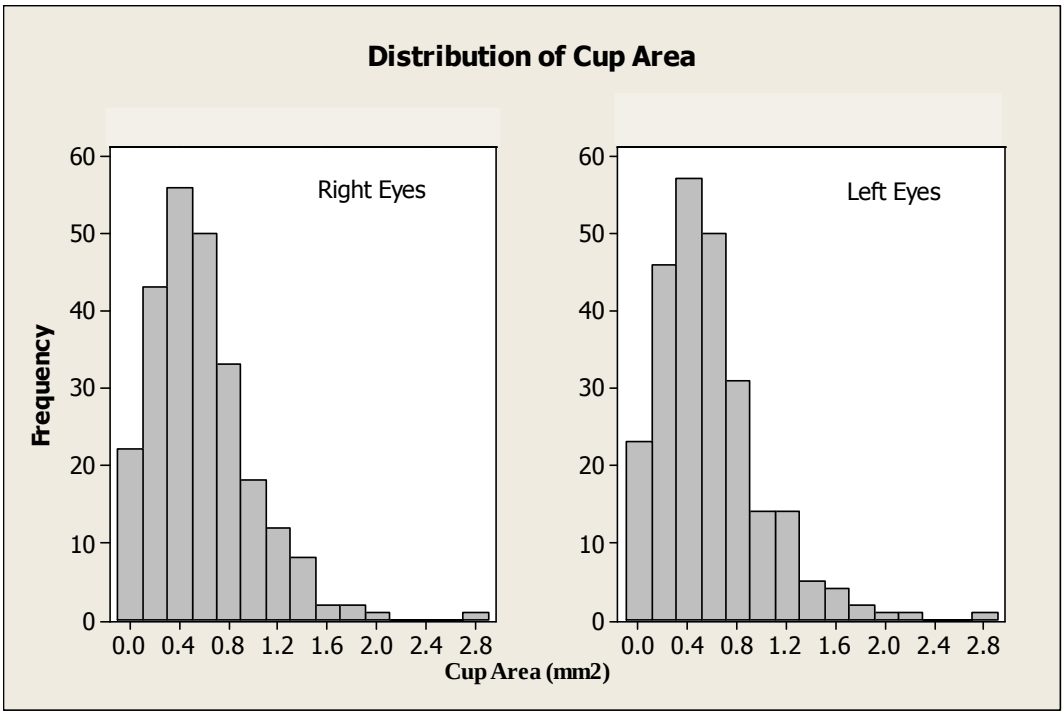

FIGURE 8.3: DISTRIBUTION OF OPTIC CUP AREA

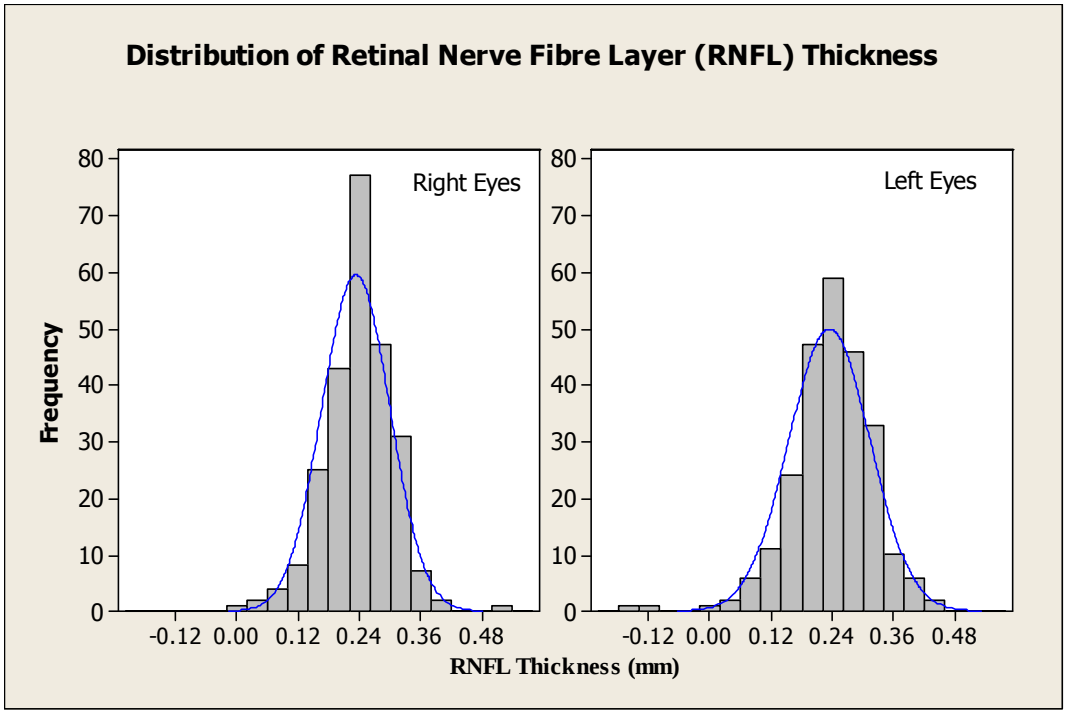

FIGURE 8.4: DISTRIBUTION OF RETINAL NERVE FIBRE LAYER THICKNESS

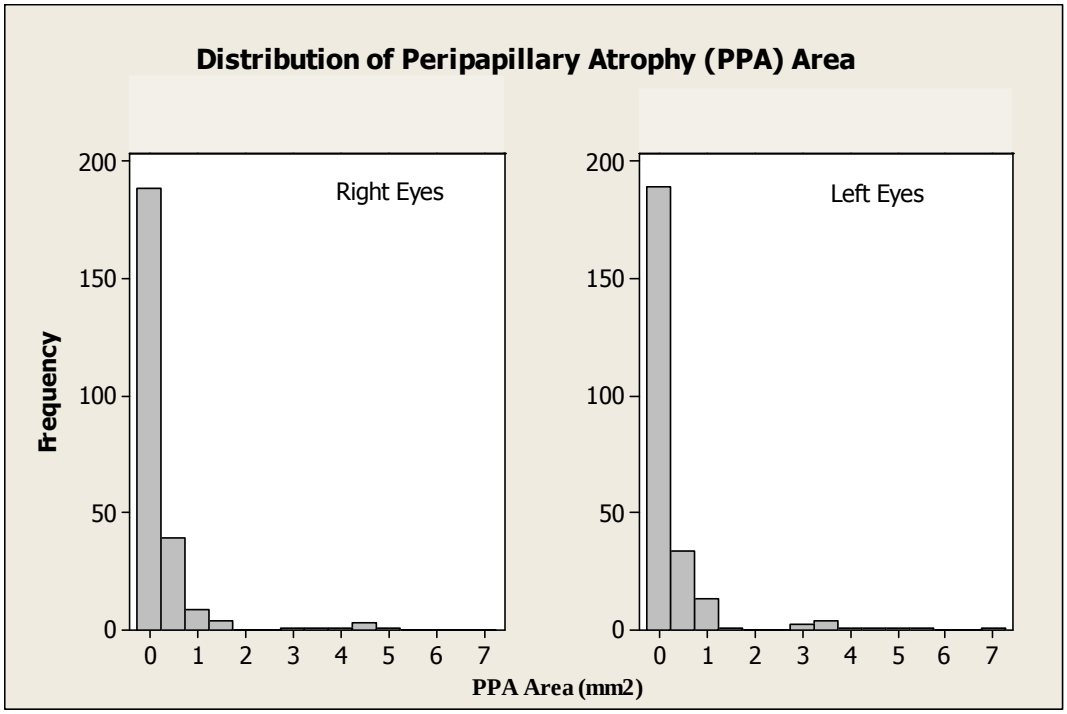

FIGURE 8.5: DISTRIBUTION OF PERIPAPILLARY ATROPHY AREA

Distributions of the above parameters were unimodal (figures 8.1 to 8.5). No statistically significant association was found between age and the optic nerve head parameters of disc area, rim area, cup area, maximum cup depth, nerve fibre layer thickness or peripapillary atrophy. A multivariate model confirmed statistically significant associations between global disc area, rim area and cup area (all p values = 0.000). Total disc area, cup area and nerve fibre layer thickness were also related to maximum cup depth (p = 0.18, p = 0.000, p = 0.000 respectively). Nerve fibre layer thickness is related to cup area, rim area and maximum cup depth (p=0.044, p=0.029, p=0.000 respectively). The area of peripapillary atrophy is related significantly to the radial extent of the atrophy (p=0.000). No statistically significant relationships were noted between total

disc area, rim area, cup area, nerve fiber layer thickness and the ocular biometric parameters of intra-ocular pressure, refractive error, axial length, central corneal thickness, anterior chamber depth or corneal curvature (all p values greater than 0.05).

8.2 DISCUSSION

Examination of the optic nerve head and its parameters plays a central role in the diagnosis of ocular disease. This is not just in the case of primary open angle glaucoma, where structural damage to the nerve is believed to precede functional problems, but is central in the evaluation of a wide range of ocular conditions (Sommer et al., 1979, Quigley et al., 1989, Harwerth et al., 1999, Kerrigan-Baumrind et al., 2000, Wollstein and Schuman, 2008). Hence understanding the appearance of the optic nerve in a disease free population is a vital part of clinical ophthalmology. Early attempts were made by Bengtsson to quantify optic nerve head parameters using an early form of planimetry in a population based survey (Bengtsson, 1976). Since then a number of population based studies have emerged documenting optic disc parameters in different populations (table 8.2). These have shown that optic disc morphology and dimensions can show inter-population, intra-population and intra-individual variation. Comparison of these studies are plagued by the same problems of comparing other eye studies, that of lack of homogeneity of methods. The early days of optic disc evaluation consisted of visual inspection (Reeves and Taylor, 2004). The introduction of ophthalmoscope by Helmotz in 1851 made the evaluation of the optic nerve head in vivo possible. However, even

with the use of more sophisticated optical equipment, reproducibility of various forms of subjective analysis of the optic nerve head shows high inter and intra-observer variability (Varma et al., 1992). More objective methods of documenting the optic nerve head have arisen (Kwartz et al., 2005, Zangwill and Bowd, 2006). Stereoscopic photographs of the optic nerve head are common a method of documenting the appearance of the optic disc but is subjective and dependent on the operators' experience and skill (Tielsch et al., 1988). Methods of planimetry have been used to quantify disc and cup area in optic nerve photographs (Spencer et al., 1995, Bourne et al., 2008a, Bourne et al., 2008b). The observer manually delineates the margins of the optic disc and cup. Computer software digitizes the image and calculates the required parameters. Optic nerve head analyzers (ONHA) such as the device produced by Rodenstock, use a video camera which takes multiple images of the fundus (Gramer and Siebert, 1989). By taking images of the optic nerve head from different angles and using a pattern of stripes projected onto the optic nerve, the ONHA is able to develop a topographic image of the optic nerve head. Even more sophisticated imaging devices are now available such as optical coherence tomograhy (OCT), which uses a reflectance technique similar to the method used in the Carl Zeiss IOLmaster and scanning laser polarimetry which uses the phase shift or retardation of polarized light as it passes through the peripapillary retinal nerve fiber layer to estimate it's thickness (Burgoyne, 2004). These methods have their own advantages and disadvantages (Gramer and Siebert, 1989, Burgoyne, 2004, Meyer and Howland, 2001). It is important to note, that with all these techniques, the values given for optic disc parameters (the "true retinal size") are computed by taking into account various magnification factors of the eye and optical system of the machine

(Rudnicka et al., 1998). A number of studies have observed that the size of optic nerve parameters can show systematic variation depending on the measurement technique (Spencer et al., 1995, Rudnicka et al., 1998, Meyer and Howland, 2001). Optic nerve measurements obtained by using the Heidelberg Retinal Tomograph, for example, have been observed to be smaller than those using photography techniques (Spencer et al., 1995, Meyer and Howland, 2001). Hence comparison of values between these studies is even problematic.

TABLE 8.2: SELECTED STUDIES INVESTIGATING OPTIC NERVE HEAD PARAMETERS

*(**ODA**: OPTIC DISC AREA; **ORA**: OPTIC RIM AREA; **OCA**: OPTIC CUP AREA; **VCDR**: VERTICAL CUP TO DISC RATIO; **NFLT**: NERVE FIBER LAYER THICKNESS; **PPA**: PERIPAPILLARY ATROPHY)*

STUDY	YEAR	METHOD OF MEASUREMENT	AGE (mean±SD or range)	POPULATION	OPTIC NERVE PARAMETERS					
					ODA (mm^2) [mean±SD]	**ORA** (mm^2) [mean±SD]	**OCA** (mm^2) [mean±SD]	**VCDR** [mean±SD]	**NFLT** (mm) [mean±SD]	**PPA** (mm^2) [mean±SD]
Erlangen, West Germany (Jonas et al., 1988)	1988	Planimetry	42.7±19.6	White Caucasian	2.69±0.70	1.97±0.50	0.72±70	0.34±0.25		
(Chi et al., 1989)	1989	Rodenstock Optic Disk Analyzer	18-35	African Caribbean White Caucasian	2.15±0.045 1.73±0.061	1.27±0.05 1.18±0.04				
(Mansour, 1991) Not population based	1991	Planimetry	-	African Caribbean White Caucasian	3.33±0.12 2.66±0.11					
Balitmore Eye Survey (Varma et al.)	1994	Topcon Image Analyzer	40+	African Caribbean White Caucasian	2.94±0.74 2.63±0.46	1.90±0.35 1.92±0.35	1.04 0.71	0.56 0.49		
(Tsai et al., 1995) (not population based)	1995	HRT I	19-40	African Caribbean White Caucasian	2.67±0.44 2.40±0.28					
Rotterdam Eye Study, The Netherlands (Ramrattan et al., 1999)	1999	Planimetry	55+	White Caucasian	2.42±0.47	1.85±0.39	0.57±0.34	0.49±0.14		

TABLE 8.2 *(CONTINUED)*: SELECTED STUDIES INVESTIGATING OPTIC NERVE HEAD PARAMETERS

*(**ODA**: OPTIC DISC AREA; **ORA**: OPTIC RIM AREA; **OCA**: OPTIC CUP AREA; **VCDR**: VERTICAL CUP TO DISC RATIO; **NFLT:** NERVE FIBER LAYER THICKNESS; **PPA:** PERIPAPILLARY ATROPHY)*

STUDY	YEAR	METHOD OF MEASUREMENT	AGE (mean±SD or range)	POPULATION	OPTIC NERVE PARAMETERS					
					ODA (mm^2) [mean±SD]	ORA (mm^2) [mean±SD]	OCA (mm^2) [mean±SD]	VCDR [mean±SD]	NFLT (mm) [mean±SD]	PPA (mm^2) [mean±SD]
(Sekhar et al., 2001) On	2001	Planimetry	34.9±13.5	South Asian	3.37±0.68	2.8±0.53	0.57±0.34	0.37±0.09		
Vellore, India (Jonas et al., 2003b)	2003	Planimetry	47.5±8.7	South Asian	2.58±0.65	1.60±0.37	0.98±0.40	0.56±0.08		
(Akar et al., 2004)	2004	Top SS Confocal Scanning Laser Ophthalmoscope	11-77	Turkish	1.98±0.42	1.41±0.35	0.57±035	0.35±0.21		
(Durukan et al., 2004)	2004	HRT II	11-75	Turkish Male: Female:	 2.11±0.43 2.12±0.41	 1.56±0.31 1.57±0.31	 0.49±0.33 0.48±0.31		 0.21±0.08 0.25±0.07	
Birmingham, Alabama. (Girkin et al., 2005)	2005	HRT II	45.9 (Afr.Car.) 42.3 (White)	African Caribbean Right eye: Left eye: White Caucasian Right eye: Left eye:	 2.14±0.05 2.18±0.05 1.96±0.06 2.02±0.06	1.6±0.03 1.6±0.04	0.56±0.03 0.39±0.03	0.33±0.02 0.27±0.02	0.28±0.00 0.25±0.00	
Bridlington Eye Assessment Project (Vernon et al., 2005)	2005	HRT II	65-89	White Caucasian	1.98±0.36	1.52±0.31	0.45±0.35		0.23±0.07	

TABLE 8.2 *(CONTINUED)*: SELECTED STUDIES INVESTIGATING OPTIC NERVE HEAD PARAMETERS

*(**ODA:** OPTIC DISC AREA; **ORA**: OPTIC RIM AREA; **OCA:** OPTIC CUP AREA; **VCDR:** VERTICAL CUP TO DISC RATIO; **NFLT:** NERVE FIBER LAYER THICKNESS; **PPA:** PERIPAPILLARY ATROPHY)*

STUDY	YEAR	METHOD OF MEASUREMENT	AGE (mean±SD or range)	POPULATION	OPTIC NERVE PARAMETERS					
					ODA (mm^2) [mean±SD]	ORA (mm^2) [mean±SD]	OCA (mm^2) [mean±SD]	VCDR [mean±SD]	NFLT (mm) [mean±SD]	PPA (mm^2) [mean±SD]
Beijing Eye Study (Wang et al., 2006c)	2006	Planimetry	40-101	Chinese	2.65±0.57					
Tanjong Pagar (Bourne et al., 2008a, Bourne et al., 2008b)	2008	Planimetry	40+	Chinese	2.17±0.46	1.43±0.49	0.74±0.35	0.55±0.10		
Central India Eye and Medical Study (Nangia et al., 2008)	2008	HRT (model not specified)	30+	South Asian	2.25±0.51					
Chennai, India (Dacosta et al., 2008)	2008	OCT	15-67	South Asian	2.63±0.55	1.78±0.55	0.87±0.45	0.52±0.14		
Siriraj (Ruangvaravate and Neungton, 2008)	2008	HRT II	30-80	Thai	2.67	2.09	0.55		0.26	
(Abe et al., 2009)	2009	HRT II	40+	Japanese subjects	2.06±0.41	1.55±0.29	0.51±0.35		0.25±0.07	

Summary statistics for optic nerve head parameters in our study are reported in table 8.2. For the reasons stated above, it difficult to compare the values we obtained in our study for optic nerve head parameters with others published in the field for any definitive conclusions. If we compare disc area in the Orcadian population to other published studies involving white Caucasian populations using the HRT to measure optic nerve parameters, our results are not dissimilar. However, if compared to studies using techniques such as the optic nerve head analyzer, mean Orcadian parameters appear smaller – an observation which is probably due to the difference in method and has also been noted elsewhere (Spencer et al., 1995, Meyer and Howland, 2001). The term macrodisc and microdisc are used clinically to describe optic discs that are considered relatively large or small. Defined as being either two standard deviations above mean disc area, for macrodiscs, or two standard deviations below the mean disc area for microdiscs, clinically this diagnosis can be of importance as these discs can be associated with other ocular abnormalities. Macrodiscs in our study sample would be above approximately 3.13mm² and microdiscs below approximately 1.01mm².

The only statistically significant associations we found between the parameters was between global disc area, rim area and cup area, between disc area, cup area, nerve fibre layer thickness and maximum cup depth and between nerve fibre layer thickness and cup area and rim area. There was no statistically significant association found between these optic nerve head parameters and age, gender or other ocular biometric parameters. Some of these findings are supported by populations based studies but not others. The Rotterdam Study, a population based cross sectional study, for example, found that age

was not associated with any optic disc parameters but disc area and rim area was marginally smaller in women compared to men (Ramrattan et al., 1999). Disc and rim area were also found to be weakly associated to refractive error. In study carried out in Antalya however, refractive error was not associated with optic nerve parameters but age was a significant factor (Durukan et al., 2004). The reasons for these differences are probably multifactorial and will be discussed in greater depth in the final chapter of this thesis.

Our positive findings described above – a relationship between certain optic head parameters with each other, are not surprising if one considers the anatomy and development of the optic nerve. Embryologically, the optic pits, the primordia of the globe, appear bilaterally at around 22 days of gestation from neuroectoderm (Edward and Kaufman, 2003). These develop into the optic vesicles which are affixed to the telencephalon via an elongation of the neuroectoderm known as the optic stalks – the primordium of the optic nerve. At around 6 weeks of gestation, axons of the retinal ganglion cells penetrate the optic stalk. The nerve fibre layer of the eye is eventually formed by axons of the retinal ganglion cells, the optic disc rim is formed from the axons as they exit the globe, and the disc area is the sum of the primordial stalk coupled with the axons. Ocular development is a highly coordinated process and a number of genes and other factors have already been identified (reviewed by Harada et al (Harada et al., 2007)). Considering their anatomical and embryological relationship, it is unsurprising that these parameters are related.

Para or peri papillary (PPA) represents chorioretinal atrophy adjacent the optic nerve head (Jonas, 2005). Commonly, PPA is divided into two zones, a zone alpha and a zone beta. Zone beta lies adjacent to the optic disc or the peripapillary scleral ring, and is characterized ophthalmoscopically by discernible sclera and large choroidal vessels. Sandwiched between zone beta or the peripapillary scleral ring (of Elschnig) and the retina lies zone alpha, a region characterized by varying degrees of hyper and hypopigmentation and areas of chorio-retinal atrophy. Histologically, the beta zone corresponds to an area characterized by a reduction in the numbers of photoreceptors and loss of retinal epithelial cells (Fantes and Anderson, 1989, Kubota et al., 1993, Curcio et al., 2000). Since the 1970s, the evidence supporting a relationship between PPA and glaucomatous optic neuropathy has been accumulating (Primrose, 1970, Wilensky and Kolker, 1976, Nevarez et al., 1988, Buus and Anderson, 1989, Jonas et al., 1992, Jonas and Xu, 1993, Jonas et al., 1989, Jonas and Naumann, 1989, Araie et al., 1994, Park et al., 1996, Park et al., 2001, Lee et al., 2002). Both alpha and beta zones have been found to be greater in eyes with glaucoma compared to normal eyes (Jonas et al., 1989, Jonas, 2005). Alpha and beta zones have been found to tally with relative and absolute scotomas respectively in visual field analysis (Jonas et al., 1991). Very recently, beta zone PPA has been identified as a risk factor for glaucoma progression as well (Teng et al., 2010). The mechanism by which PPA contributes to glaucomatous optic neuropathy is unclear but engineering models of optic nerve head biomechanics have demonstrated that the susceptibility of the optic nerve head to IOP induced stress can be influenced by the thinness of peripapillary sclera as well as the dimensions and geometry of the optic canal (Burgoyne et al., 2005). Mechanical stress induced by IOP is

greater for a smaller radius of curvature and thinner peripapillary sclera. Based on this evidence we had postulated that the extent of PPA would be related to corneal thickness - greater levels of PPA associated with thinner corneas, as well as longer axial lengths. Furthermore, despite this clinical interest in PPA as a risk factor glaucoma, there have been few population based studies investigating the distribution of PPA in a normal population.

We found the mean area of peripapillary atrophy in the right eye to be 0.24mm² (0.14-0.33mm²) and the left eye 0.28mm² (0.17-0.39mm²). The overall prevalence of peripapillary atrophy was 25.0% in right eyes and 25.3% in left eye. 9.6% of effected right eyes, and 9.5% of effected left eyes were unilateral. The only association we between the total area of peripapillary atrophy and other ocular biometric parameters was between the total area and the radial extent of PPA. Jonas et al published one of the first major studies investigating PPA in a non-glaucoma population recruited from a hospital in Erlangen, Germany (Jonas et al., 1989, Jonas and Naumann, 1989). Mean age was approximately 44 years with a range of 3-81 year. Using planimetric methods, they reported a prevalence of 15.2% beta zone atrophy in normal eyes. Mean beta zone PPA was found to 0.13mm. The Rotterdam Study is one of the few population based studied which have investigated PPA (Ramrattan et al., 1999). Using a form of planimetry to quantitate stereo-photographs, the study found 55% of their study cohort had at least one eye affected by PPA, with 13% having at least one eye affected by beta zone PPA. The prevalence of zone beta was found to increase with refractive error but no association was found with age or gender like in our study. The other population based

study to investigate PPA in the Beijing Eye Study, a cohort study in northern China (Wang et al., 2008). Beta zone PPA was found in 19.9% of volunteers in this study with a mean value of 0.46mm². Like the findings of our study, PPA did not vary significantly between genders. However, the Beijing Study did find an association between PPA and age and myopic refractive error. As before, because of the difference in methods used, sample profiles study comparison is problematic. As discussed above, "true size" of PPA is calculated by the different imaging systems using different criteria which are not necessarily comparable (Rudnicka et al., 1998, Meyer and Howland, 2001). It is however worth noting that our sample had a considerably higher overall PPA compared to the Beijing and the Rotterdam Studies (Ramrattan et al., 1999, Wang et al., 2008).

CHAPTER 9

DISCUSSION

In the early days of ophthalmology, our knowledge of the eye was initially based on visual inspection of individual cases and examination of cadaveric specimens. With the advancement of technology and research methods, further knowledge about the eye was accrued from studies. Initially these were small scale and on predominantly White Caucasian populations. These conclusions were then extrapolated to manage ocular diseases of other more varied populations. Over the past decade, but especially over the last 3 years, there has been an increasing number of population based studies looking at the prevalence of glaucoma and various QTs associated with it. The Orcades Eye Study is the first population based study investigating quantitative traits related to primary open angle glaucoma in a Scottish population. Our study has demonstrated that the average values, distribution and relationships between the primary open angle glaucoma related quantitative traits of intraocular pressure, central corneal thickness and a number of optic nerve head parameters is not dissimilar to other populations of White Caucasian descent (tables 6.1,7.1 and 8.1; figures 6.1, 7.1, 8.1 to 8.4;). In brief, we found mean IOP for right eyes to be 14.8mmHg (95% confidence intervals 14.4mmHg, 15.2mmHg), and for left eyes 15.1mmHg (95% confidence intervals 14.7mmHg, 15.6mmHg). Mean CCT in both eyes was found to be 540µm (95% confidence interval 536µm, 544µm). Mean

values for the main optic nerve head parameters with their 95% confidence intervals in brackets are as follows: optic disc area was 2.10mm² (2.03-2.16mm²) in right eyes and 2.04mm² (1.97-2.11mm²) in left eyes; mean optic rim area was 1.53mm² (1.48-1.58mm²) in right eyes and 1.48mm² (1.43-1.53mm²) in left eyes; mean cup area was 0.57mm² (0.52-0.62mm²) in right eyes and 0.56mm² (0.51-0.61mm²) in left eyes; mean vertical cup to disc ratio in both eyes was 0.39 (0.36-0.42); mean nerve fibre layer thickness in both eyes was 0.23mm² (0.22-0.24mm²). The overall prevalence of peripapillary atrophy was 25.0% in right eyes and 25.3% in left eyes. 9.6% of effected right eyes, and 9.5% of effected left eyes were unilateral. Mean peripapillary atrophy area in right eyes was 0.24mm² (0.14-0.33mm²) and in left eyes 0.28mm² (0.17-0.39mm²). The difference between left and right eyes and between genders was not statistically significant except in the case of IOP. Though the difference between left and right eyes was statistically significant for IOP at the 5% level (paired t-test, p-value = 0.021) it was not clinically significant (mean difference = 0.311 mmHg, 95% confidence interval for mean difference -0.576,-0.046mmHg). Bivariate analysis only found a statistically significant association between IOP and CCT (p=0.004) and IOP and age (p=0.005), but not with any of the other ocular biometric parameters including axial length or any optic nerve head parameter. On multivariate analysis, age and central corneal thickness were found to be associated with IOP at the 5% level (P = 0.032 and P=0.004 respectively).

Comparison of these studies is hindered by the differences of methodology used to recruit volunteers, measure QT's and define disease. Many of the techniques used to measure QTs are not comparable and have been discussed in greater detail in previous

chapters. Another problem with a number of these studies which was mentioned briefly in chapter 1, is that of self reported race. Though this remains a concept which is often disputed, race/ethnicity/population are useful constructs and indicators of ancestry for exploring hypotheses about genetic and environmental risk factors in health and disease (Burchard et al., 2003, Bamshad et al., 2004). Individuals within the same "race" tend to share a greater proportion of their genetic background as they have a more recent common ancestry than individuals from more distant populations. Recent studies investigating ancestry with more recent genetic markers have demonstrated that it is possible to assign individuals into geographic regions using certain markers (Bamshad et al., 2004). Further more, the demarcation between quantitative traits, which may have been influenced by natural selection may be greater than the difference between more neutral markers. However, for any conclusions to be valid, a study participant's population of origin must be identified as accurately as possible. The majority of studies done in ophthalmology, have relied on self reported. Unfortunately, there can be a high level of disparity between the physical appearance of a subject and any genetic measure of ancestral population (Klimentidis and Shriver, 2009). This is especially problematic in studies done in the United States where classification systems are sometimes broad and involve populations with potentially high levels of admixture. For example, the term "Asian" is used as a "racial" or "ethnic" category. However, this term encompasses South Asians, East Asians and South East Asians who are genetically and culturally a heterogeneous group. The term "Hispanic" as a "racial" or "ethnic" categorization is equally meaningless. The term is often used to described descendents from Latin American countries or descendents from Spanish cultures (Bertoni et al., 2003).

However, Hispanics are also culturally diverse and genetically heterogeneous, and can have genetic contributions from European, African and Native American populations (Sans, 2000). The contribution from each population can vary and Hispanic populations can exhibit different ancestral structures from a di- (contributions from Europe + Native American or African) to trihybrid structure (contributions from all three continents) (Bertoni et al., 2003). Admixture is also present amongst self reported White Caucasians (Halder et al., 2009). Admixed populations can also incorrectly estimate the contribution of ancestral populations to their final genetic composition (Klimentidis et al., 2009). In newer studies carried out in cohorts composed of (what we could possibly presume) to be more genetically homogenous individuals, such as populations in rural India or Mongolia, self reported race is probably less problematic.

Despite the limitations of these studies, they are important for a number of reasons. They have highlighted that primary open angle glaucoma is a heterogeneous disease, with a prevalence that varies between populations. For example, the prevalence of POAG in certain populations of African-Caribbean descent has been found to be much higher than many populations of White Caucasian descent (Rudnicka et al., 2006). The responses to treatment, course of the disease can also differ between populations. In certain African-Caribbean populations for example, the course of POAG tends to be more aggressive and less amenable to treatment than White Caucasian populations (Rudnicka et al., 2006). The type of glaucoma also varies between populations. Primary angle closure glaucoma, for example, is more common in populations of South East Asian descent that White Caucasian populations (Rudnicka et al., 2006). In addition, individual QTs and

their association with other physiological variables can also differ between some populations. Intraocular pressure for example, tends to be higher, and optic discs larger in certain populations of African Caribbean descent compared to White Caucasian populations (Racette et al., 2003). A number of studies have demonstrated a relationship between intraocular pressure and central corneal thickness (Ehlers et al., 1975, Whitacre et al., 1993, Doughty and Zaman, 2000), (Feltgen et al., 2001, Kohlhaas et al., 2006). However, this relationship does not hold true for all populations. In the Barbados Eye Study for example, a population based study in the Lesser Antilles, the relationship between IOP and CCT in volunteers of African Caribbean or "mixed" ancestry was not statistically significant, though the relationship was statistically significant for those of White Caucasian ancestry (Nemesure et al., 2003). IOP in most populations tends to increase with age. However, in Japanese populations, IOP decreases with age (Shiose, 1984). This phenomenon is not even observed in populations in mainland China (Xu et al., 2005). The Liwan Eye Study found that mean anterior chamber depth differed significantly in Chinese populations living in Guangzhou, Mongolia and Singapore (He et al., 2008a). Hence, populations which could be considered geographically, and possibly genetically neighboring each other, can not only have different "normal" values but QTs can have different physiological associations with other factors.

The understanding of what is "normal" or healthy is the first step in understanding what is abnormal, or diseased. What these studies, even with their limitations have demonstrated is that is "normal" differs between populations. The Orcades Eye Study is the first population based study investigating quantitative traits related to primary open

angle glaucoma in a Scottish population. Our study has demonstrated that the average values, distribution and relationships between the primary open angle glaucoma related quantitative traits of intraocular pressure, central corneal thickness and a number of optic nerve head parameters is not dissimilar to other populations of White Caucasian descent. Knowledge of such parameters in a normal population is invaluable in a clinical setting, as reference ranges are often used to triage patients and sometimes even define disease. The obvious example is of course intraocular pressure, values of which in the past have been the axis around which glaucoma management revolved. Intraocular pressure was initially assumed to take a Gaussian distribution. Mean IOP in White Caucasian adult populations, whether investigated by Schiotz tonometry or Goldman Applanation Tonometry, was fairly consistently estimated to being between approximately 15 to 16 mmHg, with a standard deviation of 2.5-2.8mmHg. (Armaly, 1965, Wallace and Lovell, 1969, Burr et al., 2007). " Normal IOP" was based on mean IOP ±2 standard deviations and 21-22mmHg was generally accepted as the upper limit of normal. So for many years an IOP greater than 21-22mmHg was considered abnormal. We now recognize that the concept of "normal IOP" is not that simple. For example, a subpopulation of patients exist, who develop glaucomatous optic neuropathy but never have IOPs recorded above 21mmHg and another population exists whose IOP remains above 21mmHg but do not always develop glaucomatous optic neuropathy. So though it is now recognize no absolute "safe" or "dangerous" value of IOP can be defined, and IOP goals for glaucoma must be individualized, IOP remains the only treatable risk factor for glaucoma and, a populations' IOP must be understood before targets can be set for an individual's IOP. A less obvious example is corneal width, which can be used to define

micro or megalocornea and define and subsequently monitor congenital glaucoma, as discussed in chapter 6. Corneal diameter measurements have also been used in to improve the accuracy of IOL power calculations in third generation formulas (Holladay et al., 1996), for estimating the size of the size of ciliary sulcus and anterior chamber width before the implantation of sulcus-fixated lenses (Price and Parker, 1997). Hence and understanding of the mean and distribution of corneal width in a population is invaluable.

Understanding the relationship between these quantitative traits also aids the clinical decision making process. Perhaps one of the most important of our findings is that intraocular pressure in our sample is correlated to both age and central corneal thickness. These findings are not universal (Nemesure et al., 2003, Shiose, 1984, Fukuoka et al., 2008, Xu et al., 2005, Wong et al., 2009). Though the management of primary open angle glaucoma no longer whirls around intraocular pressure alone, IOP remains an important risk factor and the only treatable component of the disease. So in the evaluation of subjects for glaucoma and other ocular conditions in the Orcadian population when the assessment of IOP is required, it is important to make appropriate allowances for age and corneal thickness. Though the measurement of central corneal thickness is considered good practice in the assessment of primary open angle glaucoma, taking such measurements when assessing glaucoma suspects is not universal (American_Academy_of_Ophthalmology, 2008). Important negatives in our sample include central corneal thickness and optic disc parameters do not vary with age, or with each other.

The differences between summary statistics and distributions between some populations could be attributed to differences in methodology. We believe that some of these differences in quantitative trait values can also be attributed to human genetic variation between populations as well as differences in environmental factors influencing these traits, not just at an individual level in the form of developmental plasticity, but at a population level.

Many of the limitations of our study we share with other published studies in the field. For example, in common with many other population based studies, though we have taken an average of several measurements, we have only measured each quantitative trait in one sitting. There is evidence that intraocular pressure and central corneal thickness can show diurnal as well as seasonal variation (Shiose, 1990, Doughty and Zaman, 2000). It is impractical in a large cohort to limit measurements of quantitative traits to the same time of day or season or the year. Furthermore, inter-individual variation would probably be such that individual peaks and troughs are likely to occur at slightly different times of the day, and 24 hour measurements of quantitative traits would be impractical and probably unpopular amongst the study cohort.

This initial analysis of data has primarily an epidemiological one and hence the ascertainment strategy of our study may be considered to be a limitation. As our results have shown, the age and gender distribution of our study does not completely mirror the age distribution of the Orcadian population (table 5.1). The long term goal of the

Orcades Eye Study is not epidemiological research but to investigate the inheritance of quantitative traits related to primary open angle glaucoma in the population isolate of Orkney. To establish this project, we utilized the resources and volunteers of the cross sectional family based genetics study, the Orkney Cardiovascular Disease Study. Hence our recruitment strategy needed to be in agreement with the strategy of the overall Orcades Project. Volunteers who had been the first to participate in other arms of the study were the first to be invited to take part in the Orcades Eye Project. There were two main reasons for employing this strategy. The first was to avoid volunteer fatigue as these individuals would have been exposed to a long succession of investigations in the other arms of the project, and we hoped to leave as much time as possible between visits. These volunteers were also the most likely to have genome-wide scans completed in the time frame of the PhD.

The second limitation of our study is the sample size of the study. In accordance with our initial power calculation, we had aimed to collect data on quantitative traits related to primary open angle glaucoma from a minimum of 1000 volunteers. Unfortunately a number of practical and logistic problems we did not anticipate forced us to defer the start date of the project and other issues impeded its progress. These were discussed in detail in chapter 5. In brief, protracted negotiations with the vendor, delayed renovations due to a shortage of appropriate manpower and inclement weather all delayed the start of the project. The progress of the project was impeded by equipment related issues, volunteer and staffing problems. Finally, it was not possible to use all the volunteer data which was gathered as only a limited number had genome-wide scans completed in time

for analysis. With hindsight, our original aims – to secure funding, find and renovate premises, source appropriate equipment, train in the use of that equipment, establish and refine standard operating procedures, set up, refine and verify databases, recruit volunteers, then gather both quantitative and qualitative data from 1000 volunteers, enter and analyze this data within the time frame of a PhD, was unrealistic. Though we have not procured the quantity of data we had initially planned, the quality of data is excellent, and the procedures and protocols which were established for data collection, entry and management, once initial faults had been resolved, have worked extremely well.

We had also hoped to calculate heritabilities for these parameters. Genetic data for the Orcades project was only accessible to a small number of individuals, so we were dependent on other members of the team to execute this analysis. A partial preliminary analysis has been performed but due to time and manpower pressures this is not complete, and now must be considered future work. Within this current data set we also have data on refractive error, axial length, corneal power and anterior chamber depth. A number of population based studies have investigated the prevalence and distribution of refractive error, a common cause of reversible visual impairment (Hyams et al., 1977, Taylor, 1981, Hashemi et al., 2004, Xu et al., 2007a, Rein et al., 2006, Resnikoff et al., 2008, Warrier et al., 2008, Casson et al., 2007, Wong et al., 2000, Wensor et al., 1999, Tielsch et al., 1995a, Kempen et al., 2004, Olsen et al., 2007, Xu et al., 2009, Wu et al., 1999, Aine, 1984, Sawada et al., 2008, Attebo et al., 1999, Wong et al., 2003). Anterior depth is an important quantitative trait related to glaucoma and has been investigated by

a number of population based studies (Aung et al., 2005, He et al., 2008a, Foster et al., 1997, Tananuvat and Pansatiankul, 2005, Hashemi et al., 2009a, Bonomi et al., 2000a, He et al., 2008b) Other future work with this current data would involve investigating the distribution and relationships of these traits in the Scottish Population Isolate of Orkney. Despite the limitations of this study, to date, the only other population based study which has collected such a wide range of POAG associated quantitative traits including peripapillary atrophy and central corneal thickness is the Beijing Eye Study (Jonas et al., 2009).

CHAPTER 10

FURTHER WORK

Though my part in data collection is now complete, there are plans for further collection of POAG associated quantitative trait data. With increased numbers, future work includes a meaningful analysis of the heritability of these quantitative traits, the use of association and linkage methods to explore the inheritance of quantitative traits related to primary open angle glaucoma. Furthermore, since this project was established, our understanding of complex disease and of the challenges of genome-wide studies and the technology to support such studies has grown. Though genome wide association studies have successfully identified a number of disease susceptibility loci, these loci only explain a small proportion of possible phenotypic variability (Manolio et al., 2009). Height for example, has an estimated heritability of 80-90% and despite a number of large scale studies identifying over 40 height associated genetic variants, these variants account for only around 5% of variation (Gudbjartsson et al, 2008, Lettre et al, 2008, Maher, 2008, Weedon et al, 2008) . Findings from other quantitative traits and complex diseases, despite high estimated heritabilities have been equally disappointing (Maher B, 2008). Several suggestions have been made to explain the limited success of current genome-wide association studies (de los Campos et al., 2010, Manolio et al., 2009). Previously utilized chips may not have captured sufficient genetic diversity, both in quantitiy and quality. Poor modeling, current additive models of

quantitative traits or complex disease not accounting for certain effects such as epistasis, or the role of copy number variations or rare variants in complex disease and quantitative trait aetiology, and inadequate sample size are other factors which may contribute to lack of GWAS success.

In the light of this knowledge, other future work and improvements to the project would include: (1). Improving genome wide coverage by using more recent methods of genotyping. Commercially available chips now not only show increased converage, they also have the ability to detect copy number varation. The Illumina HumanOmni5 Bead chip for example, has over 4,300,000 makers and CNV capability (Illumina, 2011). This is compared to 317,000 tag SNPs of the the Illumina HumaHap300 (Illumina, 2006) used in this study; (2) Increasing sample size. This could be accomplished in absolute terms by recruiting more Orcadians into the study , but considering the total population of Orkney is limited to around 20,000 individuals and even a study of 30,000 was able to identify only a very small minority (less than 5%) of variants responsible for human height (Gudjartsson et al., 2008, Maher 2008), a more pragmatic approach would be to collaborate with other research groups. Pooling and meta-analyses of genetic data by international consortia have already successfully identified new susceptibility alleles (Park et al, 2010, Schunkert et al., 2011). Collaborations between the Orcades study and a number of other population based studies has already being established (Vitart et al, 2010); (3) Analyzing other models and exploring the contribution of other factors such as the role of epigenetic variation to complex disease aetiology. Hence future work

would also include exploring the contribution of epigenetics to ocular quantitative traits (Rakyan et al., 2011).

Since this study was established, a number of studies in other populations have published heritability estimates, identified regions of the genome and putative genes for glaucoma associated quantitative traits including central corneal thickness, intraocular pressure and optic nerve head parameters including peripapilllary atrophy (Macgregor et al., 2010, Charlesworth et al., 2010, Zheng et al., 2008, Zheng et al., 2009, He et al., 2008d, He et al., 2008c, Healey et al., 2008, Healey et al., 2007, van Koolwijk et al., 2007, Carbonaro et al., 2008, Duggal et al., 2007, Tsai et al., 2009, Klein et al., 2009). These results support the use of a quantitative trait approach to locating genes and regions of the genome associated with primary open angle glaucoma susceptibility. Data from the Orcades Eye Study has recently helped identify novel loci associated with corneal thickness (Vitart et. Al., 2010). Hopefully, with further analysis and collaboration, the Orcades Eye Study will identify other genes and regions of the genome associated with a susceptibility to primary open angle glaucoma, findings which may eventually lead to the development of more effective methods of treatment for this heterogeneous and complex disease.

APPENDIXES

APPENDIX 1

Orkney Complex Disease Study (ORCADES)

Participant Information Sheet - ORCADES Eye Study (version 16/07/2007)

The ORCADES Eye Study is a new research project investigating the inheritance of an eye condition known as glaucoma. It is a part of the ongoing Orkney Complex Disease Study (ORCADES) which is looking into the inheritance of complex diseases like heart disease and diabetes.

Before you decide if you want to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

1. What is glaucoma ?

Glaucoma is a disease of the eye, often associated with raised eye pressure. It is the most common cause of irreversible blindness in the world and the second most common cause of blindness in Scotland. Glaucoma can be treated but very little is actually known about what causes it.

2. What is the purpose of the study ?

We would like to learn more about what causes glaucoma and other eye diseases, in particular to what degree genes influence the risk of disease. Hopefully this will lead to the development of better methods of treating and diagnosing such diseases in the future.

3. What will happen at the clinic ?

You will be invited to attend our eye clinic at 9 Victoria Street in Kirkwall. You will be asked questions about your health and any medications you take. The examination is similar to the type carried out by an optician and includes the following: your eyesight will be tested by reading down a chart of letters similar to those found at opticians; the pressure inside ~~your~~ eye is measured; an examination of the eye is carried out using a high powered microscope called a slit lamp which shines a slit-like beam of light into the eye to light it up for the operator; the thickness of the front 'window' of the eye will be measured by ultrasound – a drop of local anaesthetic is used; the length and other dimensions of your eye are measured by bouncing a light beam from them, which gives a very accurate measure, and does not require touching your eye. As the nerve which leads to the eye is damaged in glaucoma, a scanner is used to create a 3-D image of the nerve to check if there is any damage or changes in shape of the nerve head.

The examination will include putting drops in your eyes to dilate your pupils. This allows the back of your eye to be examined. The administering of eye drops is an optional part of the procedure. Blurred vision may occur for 2 to 3 hours and sometimes up to 8 hours after administering the eye drops. If you need to drive, the doctor will check that your eyesight meets the legal standard to drive. If you feel you cannot drive or return home by yourself, we can arrange for a taxi to take you home. This can be paid for by the project.

The questions and examination will take about an hour. If you have questions at any point, please feel free to ask.

4. What do I have to do ?

You will be given an appointment at the ORCADES eye clinic

Please bring a list of any medication you are taking. Information about family members who have any form of eye disease would be useful. Please ask their permission to provide the study with their details, so that we can contact them in the future to invite them to join the study.

5. What if the doctor finds something wrong with my eyes?
You will be asked if you would like to know about the health of your eyes. If the doctor finds a problem with your eyes which needs further investigation or treatment, there will be an opportunity to discuss this. Your permission is required to write to your GP or a specialist doctor for follow up.

6. What are the possible advantages of taking part ?
Your eyes will be examined by a qualified ophthalmologist (eye doctor) who may pick up a treatable eye abnormality that you are unaware of during the examination. She will check the pressure of your eyes and will be able to tell you if you are short or long-sighted. The information you provide for the study may help us identify genes associated with glaucoma which may lead to new treatments and methods of diagnosis of the disease in the future. Glaucoma is the most common cause of permanent blindness in the world, so this study has the potential to help millions.

7. What are the possible disadvantages of taking part ?
The clinic appointment takes approximately 1 hour and may require drops to be put into your eyes. If you do not want to have eye drops you do not need to agree to this part of the examination. These drops are used routinely in a hospital eye clinic and also by optometrists. The drops can sometimes cause your vision to become blurred for a few hours. Very rarely, the pressure in the eye can rise for a short time and some people can have a reaction to these drops.

If you have driven to the appointment, or need to drive afterwards, the doctor will check that you meet the visual standards to drive before you leave. If you do not, we can arrange a taxi to take you home. We will pay for the taxi. You should not operate heavy machinery or consider driving if your vision is blurred after these drops.

8. Who is organising and funding the research ?
The research is being organized by three teams under the overall leadership of Dr. Jim Wilson, who heads the ORCADES project. Dr. Brian Fleck, a consultant ophthalmologist at the Princess Alexandra Eye Pavilion in Edinburgh heads the ORCADES Eye Study. Dr. Vidarshi Karunaratne is an ophthalmologist and is the lead research fellow, who will be based in Kirkwall. This project is being funded by the Chief Scientist Office of the Scottish Executive, the International Glaucoma Association and the Medical Research Council Human Genetics Unit in Edinburgh.

To speak to someone outwith the team, Dr. Andy Pyott, Consultant Ophthalmologist, Department of Ophthalmology, Raigmore Hospital, Inverness IV2 3UJ, has agreed to be an independent advisor, he can be reached on, 01463 706140.

By taking part in this study, as well as receiving valuable information about your own health, you will be contributing to improving the health of the community in Orkney and in Scotland through medical research. I do hope you will take part.

Dr. James Flett Wilson
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APPENDIX 2

ORCADES

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PHONE: +44 (0)0131 651 1643 • FAX: +44 (0)0131 650 6909
ORKNEY@ED.AC.UK

Participant ID: Version 16/07/2007

CONSENT FORM FOR RETURNING PARTICIPANTS

Title of project: Orkney Complex Disease Study (ORCADES)

Principal researcher: Dr. James Flett Wilson, University of Edinburgh

Please initial box if you agree with the statement

1. I confirm that I have read and understood the ticked information sheet(s) for the above study as indicated and have had the opportunity to ask questions. ☐
   - Bone & memory (16.07.2007) ☐
   - Eye (16.07.2007) ☐
2. I agree that my GP or other medical specialist may be contacted in connection with research findings if further investigations are necessary. ☐
3. I agree to take part in the following parts of the ORCADES study: (please tick) ☐
   - ORCADES Bone & Memory Study ☐
   - ORCADES Eye Study ☐

Name of participant _____ Date _____ Signature _____

Address of participant _____

Name of person taking consent _____ Date _____ Signature _____

APPENDIX 3

ORCADES

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PHONE: +44 (0)0131 651 1643 • FAX: +44 (0)0131 650 6909
ORKNEY@ED.AC.UK

THE UNIVERSITY OF EDINBURGH

Dear A. SAMPLE 01.01.2008

Thank you for volunteering for the ORCADES study

Your results are as follows:

Measurement	Right Eye	Left Eye
Refractive Error by Autorefraction (how long or short sighted you are)	-5.00	-5.25
Axial Length in mm (the length of your eye)	26.01 mm	26.02 mm
Intraocular pressure in mmHg (the pressure in your eye)	16 mmHg	16 mm Hg.

Thank you for attending the ORCADES Eye clinic.

ORCA«IDCode»

APPENDIX 4

ORCADES STUDY **EYE CLINIC DATA SHEET**		ORCA«IDCode»
Location of visit:	«Clinic»	
Date of visit:	«Date»	Time of visit: «time»
Name:	**«Title» «Forename» «Surname»**	
DoB:	«DOB»	Maiden Name: «Maidname»
Sex:	«Sex»	
Address:	«Address1» «Address2» «Address3»	
Postcode:	«PCODE»	Telephone: «Phone»
GP Name:	«GPName»	
GP Address:	«GPSurgery» «GPAdd1», «GPAdd2», «GPAdd3» «GPPCODE»	Telephone: «GPPhone»

1

ORCADES STUDY **EYE CLINIC DATA SHEET**	**ORCA«IDCode»**
Consent signed for the project	Yes No
Volunteer has read information pack and wishes to take part:	Yes No
Occupation	
Date:	
Time:	
Squint	Yes No Don't Know
Type	exotropia esotropia exophoria esophoria Don't know
Amblyopia	Yes No Don't Know
Dry eyes /Acne rosacea	Yes No Don't Know
Refractive error	Yes No Don't Know
Type	Myopia Hypermetropia Presbyopia Don't Know Myopia+presbyopia Hypermetropia+presbyopia
Treatment	None Spectacles Contact lenses Refractive surgery _____
Glaucoma	Yes No Don't Know
Type	POAG PXF Don't Know
Treated	Yes No Don't Know
Treatment	
ARMD	Yes No Don't Know
Type	Dry Wet Don't Know
Treated	Yes No Don't Know
Type of treatment	Laser PDT anti VEGF Supplements Details
Previous ocular surgery	Yes No Don't Know
Cataract Extraction	Right Left Both Don't Know
Other ocular history	

ORCADES STUDY EYE CLINIC DATA SHEET	ORCA«IDCode»
Known Medical History	Yes No
Diabetes	Yes No Don't Know If yes: Type I Type II Don't Know
Hypertension	Yes No Don't Know
IHD	Yes No Don't Know
Multiple Sclerosis	Yes No Don't Know
Other	
Drugs taken	Yes No Don't Know
List drugs taken	Drug: Drug: Dose: Dose: Drug: Drug: Dose: Dose: Drug: Drug: Dose: Dose:
Allergies	Yes No Don't Know
Tropicamide	Yes No Don't Know
Fluorescein	Yes No Don't Know
Local anaesthetic/proxy	Yes No Don't Know
Social History	
Alcohol	Yes No units Occasional
Smoking	Never smoked Smoker Ex-smoker Cigarettes Cigars Pipe Other _____Cig/day for _____ years

3

ORCADES STUDY EYE CLINIC DATA SHEET	ORCA«IDCode»		
Family history of eye disease	Yes	No	Don't Know
Refractive error	Yes	No	Don't Know
Glaucoma	Yes	No	Don't Know
ARMD	Yes	No	Don't Know
Squint	Yes	No	Don't Know
	Type:		

Names of family members	Ocular Condition(s)	Contact Details
1.		
2.		
3.		
4.		

Pedigree

4

ORCADES STUDY EYE CLINIC DATA SHEET	ORCA«IDCode»					
	Right Eye			Left Eye		
VA Unaided						
VA Corrected						
FDT	Yes	No	Notes	Yes	No	Notes
If 'No' reason						
If 'yes'	Normal	Abnormal	Equivocal	Normal	Abnormal	Equivocal
Autorefraction	Yes	No	Notes	Yes	No	Notes
If No Reason						
Sphere (RF10)						
Cylinder (RF10)						
Axis (RF10)						
PD						
Axial Length	Yes	No	Notes	Yes	No	Notes
Corneal Curvature	Yes	No	Notes	Yes	No	Notes
ACD	Yes	No	Notes	Yes	No	Notes
White-to-White	Yes	No	Notes	Yes	No	Notes
IOP	Yes	No	Notes	Yes	No	Notes
If No Reason						
IOP 1						
IOP 2						
IOP 3						
Dilated	Yes	No	Notes	Yes	No	Notes
If no reason						
HRT III	Yes	No	Notes	Yes	No	Notes
If No Reason						

ORCADES STUDY EYE CLINIC DATA SHEET	ORCA«IDCode»	
	Right Eye	Left Eye
Central Corneal Thickness	Yes No Notes	Yes No Notes
If No Reason		
Central Corneal Thickness		
Standard Deviation for CCT		
Minimum CCT		
Maximum CCT		
Number of readings		
Volunteer Examination Subjective	Right Eye	Left Eye
Lids	NSA Other	NSA Other
Conjunctiva	NSA Bleb Other	NSA Bleb Other
Cornea	NSA Pigment Other	NSA Pigment Other
Anterior Chambers	NSA Pigment Other	NSA Pigment Other
Iris	NSA Iridotomy Iridectomy Rubeosis Other	NSA Iridotomy Iridectomy Rubeosis Other
Lens	NSA PXF Early Los NS Cortical ASC PSC Other	NSA PXF Early Los NS Cortical ASC PSC Other

ORCADES STUDY EYE CLINIC DATA SHEET	ORCA«IDCode»	
Vitreous	NAD Other	NAD Other
Retina	NAD Other	NAD Other
Optic Disc		
Disc Shape	Round Vertically Oval Horizontally oval Less regular Tilted Other	Round Vertically Oval Horizontally oval Less regular Tilted Other
Vertical Cup Disc Ratio	0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 Other	0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 Other
Disc Haemorrhage	Yes No Notes	Yes No Notes
Bared circumlinear vessels	Yes No Notes	Yes No Notes
Peripapillary atrophy	Nasal Sup-Nas Inf -Nas Temp Sup-Tem Inf-Tem Circum **None**	Nasal Sup-Nas Inf -Nas Temp Sup-Tem Inf-Tem Circum **None**
Retinal Nerve Fibre Layer	Normal Abnormal Equivocal Not Dilated /difficult to assess Other	Normal Abnormal Equivocal Not Dilated /difficult to assess Other

Other Comments/Notes	Yes	No	Yes	No
Referred ?	Yes	No		

8

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